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**Understanding the impact of food-associated stimuli on intake
in humans.**

By

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University of Sussex

Thomas Ridley-Siegert

Doctor of Philosophy in Psychology

Understanding the impact of food-associated stimuli on intake in humans.

Summary

Environmental food-associated stimuli potentiate feeding in non-human animals and humans. However, there has been no investigation of this phenomena in human adults using novel stimuli which are then selectively associated with food through different learning processes. The aims of this thesis were twofold; firstly, to investigate whether Pavlovian cues (those that signal what and *when* an outcome will occur) and discriminative stimuli (those that signal *whether* an outcome will occur) which are associated with food will increase subsequent intake. Secondly, to investigate neural activity in response to these stimuli.

The first set of studies examined Pavlovian cue-food associations. Study one utilised a new methodology to associate stimuli with specific tastes and demonstrated evidence for cue-potentiated feeding: people ate more in the presence of cues associated with a sweet taste (CS+). This potentiation was greater for foods which were sweet and so consonant with the trained taste. Study two utilised the same methodology but now contrasted sweet-likers and sweet-dislikers. However, the cue-potentiation finding failed to replicate. The second set of studies examined cues associated with the chance to obtain food-rewards, interpreted as discriminative stimuli (DS). Study three trained participants to associate stimuli with obtaining food-rewards if the correct response was produced. The findings displayed a suppression of intake in the presence of a stimulus associated with not obtaining rewards compared to a stimulus associated with obtaining chocolate rewards. Study four extended Study three however sated half the participants prior to the intake test. However the previous cue-suppression finding did not replicate. Study five examined how these two different cue-food associations

are encoded in the brain using fMRI. Analysis revealed that the stimuli modified activity in neural regions associated with reward, although whereas the DS enhanced striatal activation, the CS+ deactivated the striatum. The evidence for the lack of contingency awareness to affect behaviour throughout the thesis is discussed.

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Chapter 4

Appendix 4.1. fMRI matrix design.

Abbreviations

CR – Conditioned Responses
 CS – Conditioned Stimuli
 US – Unconditioned stimuli
 UCR – Unconditioned Response
 BMI – Body Mass Index
 VAS – Visual Analogue Scale
 DS – Discriminative Stimuli
 fMRI – functional Magnetic Resonance Imaging
 MRI - Magnetic Resonance Imaging
 PhD – philosophy doctorate
 S-R – stimulus-response
 R-O – response outcome
 CS – conditioned stimulus
 R+ - appropriate response
 R- - inappropriate response
 H – Hull’s (1943) associative factor
 S* - biologically important event or consequence
 S – unimportant stimulus
 R-S – response-stimulus
 NAcc – nucleus accumbens
 PFC – prefrontal cortex
 VTA – ventral tegmental area
 OFC – orbitofrontal cortex
 LiCl – lithium chloride
 SRC – stimulus-response compatibility
 RRVfood – relative reinforcing value of food
 LFPQ – Leeds Food Preference Questionnaire
 LED – light-emitting display
 ANOVA – analysis of variance
 CA – contingency awareness
 SEM – standard error measurement
 SIPM – Sussex Ingestive Pattern Monitoring
 CISC – Clinical Imaging Sciences Centre

BOLD – blood-oxygen level dependent

EPI – echo-planar imaging

SE – standard error

GP – General Practitioner

SPM – statistical parametric mapping

WFU – Wake Forest University

MNI – Montreal neurological institute

Chapter 1: General Introduction

1.1. *Introduction*

The commonplace view of appetite is that regulation is dependent on homeostatic control of nutrient availability; if one uses nutrients through metabolism, for example, then internal cues trigger a state of hunger to reverse this deficit (Schwartz, Woods, Porte, Seeley, & Baskin, 2000). However, increasing worldwide obesity rate (World Health Organisation, 2011) challenge these traditional homeostatic-based appetite models, with an increased emphasis on non-homeostatic factors promoting short-term over-eating (Hill, Wyatt, Reed, & Peters, 2003). One idea is that as the environment becomes increasingly obesogenic, the near omnipresence of food and food-related cues influence eating (Ely, Winter, & Lowe, 2013). With one's environment potentially containing vast numbers of food-associated cues it is critical to understand the influence these stimuli have on consumption. This thesis will explore the effect of food-associated stimuli on ingestive behaviour in a controlled, laboratory setting, as well as the neural activity elicited by these stimuli.

How these cues come to be 'related' to food has been the interest of psychologists for over one hundred years. However, to understand current theories of how learnt associations between cues and food develop the origins of these theories need to be understood. The initial part of this review of the influence of cues on eating therefore explores the historical context for the development of current ideas of how we learn about food-related cues.

1.2. *Thorndike*

Our current understanding of the concept of reinforcement began with Edward L. Thorndike (Thorndike, 1898). His main area of investigation was whether animals could learn behavioural tasks through imitation and repetition. Thorndike is probably best known for experiments from his PhD dissertation in which animals were tasked with escaping from a puzzle box. In his own words, his methodology would be "to put animals when hungry in enclosures from which they could escape by some simple act" (1898, p.6). Specifically his experiments would typically entail placing a cat inside a box with food being visible outside. The animal would need to produce a behaviour, such as pulling a wire loop, pulling a lever or pressing a treadle, in order to escape from the box and obtain the food. Thorndike repeated this process, continually measuring the time it took the animal to escape. He found that animals took progressively less and less time to escape the box as the number of trials increased. They had seemingly learnt

what behaviours they needed to perform to escape from the box; they had formed an association between the stimulus situation and the appropriate response.

In this original trial-and-error learning experiment Thorndike identified two features that were necessary for learning; firstly that the cat needed to be hungry otherwise it might not have escaped the box. Unfortunately Thorndike never empirically tested this, he merely speculated. Interestingly, his conclusion was drawn despite his observation that the cat “does not pay very much attention to the food outside, but seems simply to strive instinctively to escape from confinement” (1898, p. 13). Whether the motivation was the motivation to escape the confinement of the box or to reach the food, Thorndike was introducing the idea of drive (although he never used that specific term). Thorndike’s second observation was that learning consisted of the strengthening of a connection between a stimulus situation and a response. Any response which was followed by what he called “satisfaction” would become attached to the situation and would be more likely to occur if that situation arose again. He called this the Law of Effect. This connection would be strengthened if the response produced a satisfying effect for the animal or would be weakened if the response produced a discomforting effect.

1.3. Watson

At the time of his work, the biggest, and most public, critic of Thorndike was John B. Watson (See Bolles, 1979). Watson’s associationist idea (1913; 1930) was simple; similar to Thorndike’s work, learning consists of strengthening Stimulus-Response connections or habit (S-R). When an animal received a reinforcer, such as food or drugs, it would cause an increase in the strength of the S-R association that immediately preceded it. Whatever behaviour was produced immediately prior to receiving the reinforcer was the strengthened response; he called this the Law of Recency. However, in his formulation, there was no expectation of reward, nor any hedonic or affective state produced by the reinforcer. The strengthening of the S-R could occur directly. The stimulus produces activity in one brain area, the response another; when these two areas are activated at the same time the neural pathways between the two are strengthened. The strength of the association would not be dependent on the effect it produced (magnitude or value of reinforcer was irrelevant to Watson) but dependent on the frequency with which it occurred.

The absence of any affective experience in the production of a behaviour was an interesting concept, and in stark contrast to Thorndike’s work. To Watson it was irrelevant whether catnip (something cats love) or boring dry cat food was available to the cat outside Thorndike’s box, as long as the cat had performed the behaviour the same number of times the strength of the association would be the same for each. Evidence for this S-R habit learning is provided by

Carr and Watson (1908). In that experiment rats were trained to complete a maze in order to receive food. The rats gradually learnt the location of this food and ran quickly to that location. When the maze was lengthened the rats ran to the previous location of the food then stopped despite food being no longer present. It seemed as if they had learnt a habitual response that they performed regardless of whether they obtained a reinforcer or not.

However, the associationist theory could not explain how an individual could learn long behaviour-chains, where an animal's chance of receiving a reinforcer is dependent on multiple behaviours occurring in a specific order. Subsequently, research has shown that individuals form Response-Outcome representations (R-O) in addition to Stimulus-Outcome representations (Colwill & Rescorla, 1985a; Colwill & Rescorla, 1985b; Colwill & Rescorla, 1988). Watson's associationist theory, also, ignored the importance of drive. Drive is any strong stimulus that can arise from biological needs but are not tied exclusively to needs (Dollard & Miller, 1950). To Watson it did not matter if the cat was hungry or sated in Thorndike's box: the differing states would not change behaviour.

1.4. Pavlov

Developed at a similar time (but not translated from Russian until later) was the work of a Russian physiologist, Ivan Pavlov. Based on Rene Descartes' idea of the reflex arc, Pavlov (1927) believed that animals' behaviour is the manifestation of biological reflexes. These reflexes would be produced by the following; an external or internal stimulus activates a nervous receptor which sends a nervous impulse to the central nervous system. Based on existing nervous connections this sends an impulse through an outgoing nerve fibre to activate an organ. These reflexes might be positive or negative, excitatory or inhibitory. Pavlov believed that reflexes generally (though this was based on the two reflexes, alimentary and a mild defence reflex, he was interested in) had two distinct components; a motor and a secretory. In the alimentary reflex, for example, this would be a motor activity to obtain the food, such as walking towards the food, and a secretion of saliva.

The most important discovery by Pavlov was that external stimuli could gain control over these responses. Previously Thorndike (1898) had briefly attempted this by shouting "I must feed those cats!" and clapping his hands four times (1898, p. 75) before giving hungry cats a piece of fish and recording whether the cat climbed up the wire netting on its cage. However, to gain complete control over all extraneous variables a special laboratory was built at the Institute of Experimental Medicine in Petrograd. This was the dawn of well controlled behavioural experiments. In his most famous experiment, a dog had been stimulated by the sound of a metronome (an initially neutral stimulus) and immediately presented with food (an

unconditioned stimulus, US) which produced salivation (unconditioned response, UCR). According to Pavlov, this initially neutral stimulus had been superimposed upon the action of the inborn salivary reflex. Through repeated pairings the metronome (interpreted as a conditioned stimulus, CS) now was able to produce the alimentary response (both the salivary secretion and the motor reactions), now termed the conditioned response (CR), in the absence of the food. After this conditioning “the animal reacts to the signal in the same way as if it were food” (Pavlov, 1927, p. 22). However, there were a number of stipulations. Firstly, there were limits to what behaviours could be conditioned to certain stimuli; there must have been temporal overlap between the initially neutral stimulus and the UCR. The sound of the metronome had to occur simultaneously with the presentation of food. However, this stipulation was shown to not be necessary by Garcia, Kimeldorf and Koelling (1955). In their study of conditioned taste aversion they exposed rats to gamma radiation during exposure to a solution. Rats avoided the taste stimulus previously associated with radiation despite the radiation-induced illness occurring after exposure to the solution. Another stipulation was that a defence reaction could not be transformed into an alimentary conditioned reflex.

1.5. Hull

However, drive was clearly an important factor that was currently being overlooked. A salty pretzel might be hedonically rewarding when hungry but aversive when thirsty or sated. The first person to introduce the importance of drive state into learning theories was Clark Hull. Based on previous experiments Hull (1943) proposed that the strength of one’s behaviour is determined by the animal’s drive and the amount of prior learning:

$$E \text{ (strength of response)} = H \text{ (associative strength of habit)} \times D \text{ (drive)}.$$

First in this formula is drive. Hull postulated that drive activates and generates behaviour; no specific behaviour is produced by drive, it is merely a general motivator. In contrast habit was incredibly specific. Habit (the S-R connection) was the tendency of a specific stimulus to evoke a specific response (Hull 1943) learnt through sequences of cues (through learning the sequence backwards) by Pavlovian conditioning. All reinforcers share the ability to reduce drive. If a response resulted in a reduction in the drive then it will be known as the appropriate response (R+), whereas those inappropriate will not do this (R-). These responses are in direct competition with each other and the aggregate of these will determine which behaviour is produced (Hull, 1952). This was the first suggestion of conditioned inhibition; when a response occurs in the absence of reinforcement this response will be inhibited in

future. This important factor also allowed the formulation of discrimination learning (Hull, 1952); in the presence of one stimulus a response is produced whereas in the presence of another stimulus the same response is not. Hull proposed that hunger and thirst could become discriminative stimuli to determine whether a habit is triggered. The difference between this discriminative learning and trial-and-error learning (as in Thorndike's work) is that discrimination learning entails primarily stimulus selection, in comparison trial-and-error learning involves response selection (Hull, 1952).

The importance of Hull's theory could not be understated. This theory was a divergence from mental or physiological events and focused almost solely on behaviourism. His Popperian theory was testable and explicit (see Bolles, 1972). However, the necessity of drive was heavily criticised. According to Hull's theory, due to the multiplication of strength of habit and drive if either was zero then no behaviour would be produced. This obviously could not explain how new behaviours occurred of which there would be no strength of habit or how behaviours could occur if there was no drive. The coup de grâce to the necessity of drive was given by Olds and Milner (1954) who showed that animals and humans will learn to produce a behaviour and continue to work for a depolarizing electrical stimulation via implanted electrodes to be delivered in their lateral hypothalamus, nucleus accumbens, or other associated sites. This occurred despite this electrical stimulation not reducing drive.

However, Hull, in collaboration with Kenneth Spence, developed Hull's reinforcement theory into the original motivation model. This model included three assumptions; firstly, that incentive motivation is a motivator that multiplies existing habits. Secondly, it is based on some emotional response of the automatic nervous system. And lastly, the response produced is acquired through Pavlovian conditioning. Hull's original theory included a number of interesting ideas that remained in the incentive motivation model. Similar to Watson, there was an 'associative factor (H)' which was determined by the number of occasions that the S-R was followed by the reinforcer. Hull (1952) also introduced an incentive motivational variable which he assumed to be a function of the magnitude of the reinforcer. Spence (1960) developed his own theory in which he speculated that the number of associations made and the reinforcer's magnitude does not determine the associative factor but the incentive motivational variable. Spence argued that this variable combined multiplicatively with habit and with drive additively. Drive, according to Spence, was not just physiological drives but also emotional. Thorndike's cats may have been driven by the emotional distress of being trapped inside a box rather than hunger, or a combination of the two.

1.6. Bolles

However, Hull's drive theory had failed to explain a number of phenomena such as auto-shaping which occurred in the absence of response reinforcement. Auto-shaping (Brown & Jenkins, 1968; Williams & Williams, 1969) is when animals produced consummatory behaviours to the stimuli associated with food at the expense of obtaining the reward itself. For example, when pigeons were trained to associate an illuminated key with receipt of a food, the birds would peck the illuminated key even when pecks turned off the key and prevented reinforcement (Williams & Williams, 1969). Prior theories had failed to explain why these stimuli become so attention-grabbing that they become the goal of behaviour at the expense of obtaining food. This problem was addressed by Robert Bolles. Bolles (1972) suggested that Pavlovian conditioning occurs whenever an unimportant stimulus (S) is paired with a biologically important event or consequence (S*). This first stimulus S elicits an expectation of the second S*. These S-S* associations are learnt relatively quickly and are generally accurate representations of S-S* in the environment. In contrast Bolles suggested that instrumental conditioning occurred whenever an S* is contingent upon a particular response (R). This learning is more uncertain, takes more time and is constrained by the animal's innate response tendencies, however has been established over a large array of physiological responses, such as gastric mobility, localized changes in blood pressure, and formulation of urine (see Bolles, 1979). Thus, in most instrumental conditioning experiments animals learn both S-S* and R-S* contingencies. Bolles postulated that these two types of expectancies (S-S* and R-S*) synthesize so in the presence of S the animal would be likely to produce the response R despite animals never having paired S with R. He also importantly noted that animals could have innate R-S* expectancies. Bolles (1972) argued that the probability of a response was determined by three things; (a) the strength of the S-S* expectancy, (b) the strength of the R-S* expectancy, and (c) the value of the S*. This last factor, the value of the S*, was similar to Hull's drive but with more generality. The value of the S*, Bolles argued, would certainly depend on the physiological state of the organism. When hungry food has high value but when sated it has low value. This addition was critically important.

Bindra (1974) had previously suggested that all learning is S-S* learning and that conditioned cues elicit motivational states that generate relatively fixed responses directed at those cues. Bolles (1979) thoroughly disputed this rigidity of behaviour arguing that while this may be applicable to Bindra's work, which was based on the learning of birds (which consists of responding in old ways to new stimuli due to their limited ethology), the unpredictability of the CR and the plasticity of mammalian behaviour suggests that what the animal learns is not a fixed pattern of responding to the S but rather an expectation of the S*. This was supported by

Rescorla (1968) who showed that contingency (whether the CS predicts the US) is more critical to learning than number of associations.

However an important question remains in regards to Bolles' theory; if expectation drives behaviour then why wouldn't the animals merely wait to receive the reward rather than conduct the detrimental behaviours seen in auto-shaping, schedule-induced polydipsia (excessive thirst), etc.?

1.7. Bindra

It was Bindra (1974; 1978) who tried to explain this expectation gap. He suggested that expectancy alone could not explain the irrational behaviour seen in the previously mentioned phenomena. Bindra stated that the primary effect of the reinforcement procedure is "not response strengthening, but the creation of a motivational state that influences a wide variety of subsequent behaviour of the animal" (1969, p. 7). In other words Bindra's suggestion was that an S does not simply evoke S* expectation but evokes, what he termed, a "central incentive-motivational state" (1968, p. 7). This state was a hypothetical set of neural processes that could promote goal-directed behaviour in regards to particular classes of incentive stimuli. These stimuli take on incentive properties through Pavlovian conditioning; specifically, the S takes on specific motivational and hedonic properties of the S*. Specifically, these properties are incentive properties which attract the animal and cause goal-directed behaviour and consumption. It is these cues that trigger fixed behavioural patterns directed at the cues, and in its extreme form can lead to the animal perceiving the S as the S* (as evidenced by pigeons trying to "eat" a food-associated stimulus when hungry or "drink" a light when thirsty (Jenkins & Moore, 1973). Bindra believed that this explanation could explain auto-shaping. If the CS is presented in similar positions spatially and temporally as the US then the animal would be likely to produce the same behaviours to the CS as it would to the US.

Bindra strongly believed that the only important form of learning was S-S* learning. He believed that S-R or R-S contingencies were relatively trivial in learned behaviour modifications. Bindra (1969) argued that the reinforcement procedure had no direct effect on the response the animal produced; the procedure only establishes incentive cues. It was these cues that elicit fixed behaviour patterns or consumption, as well as motivating on-going behaviour. The response produced by the animal was determined by the properties of the stimulus and its spatial position. The motivational state does not activate a predetermined response; instead a fresh response is constructed as the animal interacts with certain environmental stimuli which have become more potent. Bindra argued that the only reason one sees uniformity of responses in instrumental conditioning experiments is due to the uniformity of the spatial and

temporal arrangement of incentive stimuli. In normal situations Bindra proposed that the reason response selection and motivation seem integrated is due to the fact that often the motivation and goal of the response (e.g. food) are determined by the same incentive object (e.g. food odour).

However, Gallistel (1978) argued that motive attribution was not enough to explain behaviour. If CSs became incentives themselves then an animal would be expected to respond to them regardless of physiological state. In fact Bindra had addressed this previously. Bindra (1969) suggested that organismic conditions are important as they serve as 'gates' or limits within which certain incentive stimuli have an effect. He argued that motivation processes are not exclusively linked to any organismic-state variable (or homeostatic drive) or to incentive stimulus properties, but to both equally. However, it is to be noted that Bindra believed that no goal-directed motivation is generated by organismic conditions per se; the manifestation of goal-directed motivational states was solely a matter of environmental incentive stimulation (Bindra, 1978).

1.8. Toates

The ideas by Bolles-Bindra, in particular their notions of S-S* associations and how CSs can gain incentive properties, were developed by Toates (1986). Toates suggested that, similar to the previously mentioned theories, CSs acquire incentive properties via Pavlovian associations. It was these hedonic incentives that were the target of motivation, not only arousing motivation but given this motivation direction (Toates, 1981). After these S-S* associations have been learnt the S can stand in for the S*. In an experiment by Holland (1990), rats paired one tone with a wintergreen-flavoured sucrose solution and a different tone with a peppermint sucrose solution. Then after pairing one of the tones with lithium chloride (to induce illness) rats were offered the two sucrose solutions. Holland found that rats drank considerably less of the solution previously paired with the tone associated with illness despite the flavour never being paired with illness. The CS had substituted for its taste. This explanation could explain the previously mentioned phenomena, such as auto-shaping or schedule-induced polydipsia (excessive thirst) (Falk, 1966; Schuster & Woods, 1966).

1.9. Cabanac

The critical clarification of Toates was that incentive value was dependent on drive state (Toates, 1986). Whilst certain substances, such as sweet tastes, may hold an intrinsic value and be innately liked, the instantaneous value may be confirmed or adjusted by the experience one has in different physiological states (Toates, 1981). Toates had developed this idea based on

the concept of alliesthesia (Cabanac, 1971, 1979, 1992). Alliesthesia is when a “given stimulus can be perceived as pleasant or unpleasant according to the inner state of the subject” (1979, p. 9). In other words, drive states have the ability to potentiate primary rewards’ hedonic value. Pleasure was not an invariant property of food thought as tasty (Berridge, 2000). For example, Cabanac and colleagues showed that pleasantness ratings for sweet stimuli were higher before ingesting a preload of glucose than after (Cabanac, Minaire, & Adair, 1968; See Cabanac, 1979). Similarly, one hour post ingestion of a 900 calorie liquid lunch normal-weight participants were shown to decrease their pleasantness ratings, as well as salivation rate, compared to when they had a lower (450) calorie meal (Wooley, Wooley, & Woods, 1975). This effect has been shown to occur in a range of different responses, such as pleasantness ratings of skin temperature changes (see Cabanac, 1979). Booth (1977, 1980) showed that the value can even change within meal. Booth gave rats a choice between two identical foods paired with different flavours (flavour 1 had been associated previously with a high-calorie diet whilst flavour 2 with a low-calorie). Initially rats preferred flavour 1 but then as the meal progressed switched to flavour 2. This was interpreted as a switch to a lower-calorie diet as satiety approached in order to avoid excessive energy intake. The food’s incentive value was not determined by the diet itself or any learned properties of it but altered depending on current energy state.

Drive states can also interact with stimuli to potentiate the value of the incentive stimulus. Toates suggested that this relationship was bidirectional. External stimuli have the ability to potentiate appetite as effectively as an increase in physiological hunger (shown in priming studies, such as Cornell, Rodin, & Weingarten, 1989). According to Toates, these incentive properties of the conditioned stimuli are regulated by drive states in the same way as the incentive properties of the food itself would be.

Toates theory predicted a number of interesting implications. The hedonic value of the food itself and stimuli are not fixed, as previously shown by Booth and others. Also, drive states can be potentiated by sudden CS experience. According to this theory, the mere smell of cookies can make peoples’ mouths water and enhance the hunger drive state. Lastly, in order for motivation to have an effect both incentive stimuli and drive are needed. Drive reduction alone is insufficient for animals to learn a new behaviour (McFarland, 1969; Miller & Kessen, 1952) and only when rats experience the oral and physiological aspects of eating do they stop eating (Bedard & Weingarten, 1989).

1.10.1. Robinson and Berridge's incentive salience theory

The Bindra-Toates model postulated that rewards have incentive value due to their ability to elicit pleasure. This implied that these conditioned incentives were equivalent to the hedonic value of the reward (Toates, 1986). This view was heavily challenged by Terry Robinson and Kent Berridge in their proposed incentive salience model (Robinson & Berridge, 1993; Berridge & Robinson, 1998; Berridge, 1996). Incentive salience is a property of stimuli associated with reward that makes the stimuli attention grabbing, attractive, wanted, and the target of goal-related behaviours (Berridge, 1996). Robinson and Berridge believed that there were three parts to the acquisition of incentive salience: Firstly, the US produces hedonic activation. Secondly, one learns the association between the CS and the US. Lastly, incentive salience is attributed to the CS. Once these stimuli have been attributed with incentive salience (Berridge [2000] also noted that the stimuli could also be attributed with hedonic properties, as in evaluative conditioning), these cues become motivational beacons for behaviour, enabling arousing effects of drive state, and can substitute for the reinforcer itself (as seen in auto-shaping).

Reward, and incentive motivation, is proposed to be made up of three distinct parts; pleasure, associative learning and incentive salience. Whilst normally these three processes act together, a large of body of evidence has shown they are separate, operated by different neural systems.

1.10.2. Liking

The first of these psychological processes is 'liking' (note. 'Liking' (in inverted commas) is the unconscious process that underlies conscious liking, since Robinson and Berridge (1993) believed that the core process was not liking in the usual sense). Pleasure is simply a triggered affective state –contrary to popular belief, there is no object of desire (Berridge, 2000). Pleasure is neither goal-directed nor necessarily associated with stimuli. 'Liking' by itself is not true reward; nothing is rewarded by hedonic activation alone (Robinson & Berridge, 1993). It is the incentive salience attribution that develops stimuli into desirable objects. 'Liking' has been shown to occur in the absence of 'wanting' ('wanting' refers to the core process produced by an incentive). It has been suggested that the neurotransmitter dopamine is responsible for mediating 'wanting' whereas opioids are responsible for mediating 'liking' (Berridge, Robinson, & Aldridge, 2009). Berridge and Robinson (1998) tested animals with extensive dopamine depletion (from 90-99.8% in the neostriatum and 74.1-99% in the accumbens) on their hedonic reactions to sweet stimuli (which are innately liked: Berridge, 1996). They found that despite this depletion, animals still showed hedonic reactions to the palatable stimuli, as well as

aversive reactions to a bitter quinine solution. Similarly, in the same paper they showed that this depleted dopamine system was not needed for the pharmacological enhancement of hedonic reactions. In the second experiment from this paper, rats with severe dopamine depletion learnt that an initially liked stimulus can become aversive after the stimulus was paired with lithium chloride. This critically showed the lack of necessity of dopamine for adjustments in hedonic value. Collectively this elegant series of experiments show the possible dissociation of 'liking' and 'wanting'.

Two important brain structures which have been suggested to contain 'hedonic hotspots', which mediate 'liking', are the Nucleus Accumbens (NAcc) and the ventral pallidum. In regards to the NAcc it has been shown that the hedonic mediating area is specific to the shell, rather than the core (Peciña & Berridge, 2000). Microinjections of opioids into specific areas of the NAcc shell have been shown to increase feeding and hedonic patterns of behavioural affective reactions to sucrose (Peciña & Berridge, 2000). Also, neuroimaging studies have shown activation in these areas during ingestion of pleasurable liquids (Berns, McClure, Pagnoni, & Montague, 2001). The ventral pallidum is the main output target of the NAcc. Microinjections of an opioid agonist into the posterior ventral pallidum caused increased hedonic 'liking' reactions to sucrose as well as causing rats to eat over twice as much food (Smith & Berridge, 2005). Interestingly, Smith and colleagues have shown that neurons in this region can code changes from 'disliking' to 'liking' that occur when rats enter a new physiological state (Tindell, Smith, Peciña, Berridge, & Aldridge, 2006). For example, ventral pallidum neural firing in response to a noxiously intense taste of seawater was doubled when rats were in a state of salt depletion compared to when they were in a normal homeostatic state. This region coded the hedonic value of the saltwater when initially it was disliked, in the normal homeostatic state, to when it was liked in the depleted state. This supports the alliesthesia concept that sensory pleasure is dependent on drive state (Cabanac, 1971). Other sites have also been implicated in coding hedonic stimuli, such as the OFC which has been shown to be activated by pleasant tastes (O'Doherty, Deichmann, Critchley, & Dolan, 2002; Small et al., 2003), and odours (Rolls, Kringelbach, & de Araujo, 2003). It may be that these distributed 'liking' sites interconnect to form a single integrated 'liking' system (Peciña, Smith, & Berridge, 2006).

1.10.3. Wanting

Similarly, 'wanting' has been shown to be a separate process, able to operate in the absence of 'liking' and associative learning. Microinjections of amphetamine in sensitized rats caused a 100% increase in lever pressing when presented with a stimulus previously associated with sucrose, compared to control rats after vehicle microinjection (Wyvell & Berridge, 2001).

This study suggests that sensitization of the dopamine reward pathway caused incentive cues to elicit excessive goal-directed behaviour. Schultz and colleagues have shown that dopamine neurons fire to food-associated stimuli to a greater extent than during actual consumption (Schultz, Apicella, & Ljungberg, 1993). The dopamine receptor blocker pimozide has been shown to reduce lever-pressing and running for food reward in hungry rats by blunting “the rewarding impact of food and other hedonic stimuli” (Wise, Spindler, De Wit, & Gerber, 1978; p. 262). However, importantly, dopamine antagonists do not alter hedonic palatability measured by tongue protrusions, paw licks and rhythmic mouth movements (Peciña, Berridge, & Parker, 1997). Stimulation of the lateral hypothalamus can elicit consumption by potentiating salience, but not the hedonic value (Berridge & Valenstein, 1991).

In contrast to the ‘liking’ system, incentive salience attribution is dependent on the mesocorticolimbic system consisting of dopamine neurons projecting from the ventral tegmental area (VTA) to the NAcc, amygdala, prefrontal cortex (PFC) and other forebrain areas (Ikemoto, 2007; Kelley & Berridge, 2002). Destruction of these mesotelencephalic dopamine projections causes substantial aphagia, causing these animals to ignore stimuli which normally induce goal-orientated behaviour (Stricker & Zigmond, 1973). Electrical stimulation of these areas, such as the ventral striatum, is highly rewarding (Ikemoto & Panksepp, 1999). The mesocorticolimbic system, though, is also activated by aversive stimuli such as stress or electric shocks (Piazza et al., 1996), suggesting a role for this system in motivation generally rather than specifically to aversive or attractive stimuli. Cues previously associated with reward have been shown to cause firing in dopamine neurons (Schultz, 1998) reflected in dopamine release in the ventral striatum, the magnitude of which correlates with incentive motivation properties of the cue (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004). However, as learning increases the dopamine neurons fire in response to the salient cues associated with reward and cease activation to the now-expected reward (Schultz et al., 1993). The predictive nature of these cues also modulates the firing of these neurons. For example, if the predicted reward fails to appear dopamine neurons are depressed at the time of reward, or if the reward appears earlier than expected dopamine neurons fire (Miller & Cohen, 2001). This suggests these neurons are coding a ‘prediction error’ (the extent to which a reward, or cue associated with reward, is surprising (Montague, Dayan, & Sejnowski, 1996; Schultz, 1998)). In humans, fMRI studies have shown similar findings to the animal research previously mentioned. The ventral striatum, into which the mesolimbic dopamine neurons project, has been shown to be critical for learning about motivationally salient stimuli, irrespective of stimuli valence, and to use that information to influence action selection (Jensen et al., 2007). Interestingly Hare and Colleagues (2008) have suggested that different areas of this incentive

salience attribution pathway may play different roles in incentive attribution. They found that goal values were correlated with medial orbitofrontal cortex (OFC) activity, decision values with central OFC activity, and prediction errors with ventral stratum activity (Hare, O'Doherty, Camerer, Schultz, & Rangel, 2008); the latter finding was supported by Montague et al., 1996).

1.10.4. Learning

Lastly, associative learning has also been shown to be separate from 'wanting' and 'liking'. Mice that are unable to make dopamine have been shown to learn to prefer a tube that delivered a sweet solution over a tube that delivered a water solution (Cannon & Palmiter, 2003). Similarly, dopamine knockout mice can learn instrumental associations equally well as wild-type mice (Yin, Zhuang, & Balleine, 2006). Mice with chronically elevated dopamine do not learn S-S* reward predictions more quickly than wild-type mice nor were their responses more persistent or stronger (Cagniard, Balsam, Brunner, & Zhuang, 2006).

Still, Berridge (2000) was keen to highlight that incentive salience attribution does not work in isolation. Bolles (1972; 1979) suggestion of a cognitive expectation of a reward for goal-orientated behaviour was suggested to operate alongside the mechanism for incentive motivation. It might seem rational to assume that the value of the reward and its incentive are held in mind whilst the actor performs an action to obtain it, however work in animals have shown that these two processes are distinctly separate. Balleine and Dickinson (1991) trained thirsty rats to press a lever for a sucrose solution in one conditioning session. The rats were then injected with nausea-inducing lithium chloride (LiCl) either immediately post training session or 6 hours later (no associations between sucrose and illness made). If the LiCl was injected immediately post training it abolished the rewarding properties of sucrose, however it did not affect the associated instrumental response. Specifically, rats who associated sucrose with LiCl-induced illness immediately post training lever-pressed to an equal frequency as control rats. However, if these experimental rats were given the opportunity to sample sucrose prior to the instrumental test, then they reduced their frequency of responding. These results appear to suggest that without experiencing a devalued reward, the rat's expectations of this reward is based on its value at time of learning. In comparison, according to the rules from the Bindra-Toates model, incentive learning can incorporate new information due to changes to physiological drive states despite not experiencing the new hedonic value in this new state. However, Rescorla (1992) has shown that rats can indeed adjust their behaviour prior to experiencing the devalued reward. In a series of experiments, rats paired one instrumental response with one outcome (pellets) and another response with a different outcome (liquid sucrose). One of the outcomes was then paired with LiCl. Rescorla showed that rats were able

to adjust their behaviour despite no re-exposure to this devalued reward. This seemed to occur due to methodological changes (such as pre-training of both responses to a different, single outcome in order to maximise vulnerability of each response when they were then paired with distinct outcomes). However, more evidence for the separation of incentive attribution and cognitive expectancy comes from animal lesion studies. Balleine and Dickinson (1998) have suggested that the prelimbic area of the prefrontal cortex is critical for contingency learning, whereas incentive learning involved the insular cortex. In a series of experiments Balleine and Dickinson (1998) trained hungry rats to associate to press a lever and pull a chain for food pellets and starch solutions respectively. The animals were then sated on food pellets or starch. The rats were then tested on their responses. However, critically during this test session one type of reward was delivered randomly once every 50 seconds without an instrumental response required. In control rats and rats with lesions to the insular cortex unpaired rewards reduced the action paired with the same reward to a greater extent than that paired with the different reward. However, for prelimbic lesioned animals the introduction of the unpaired rewards reduced performance of not only the action paired with the same reward but also paired with the different reward. This suggests an insensitivity to the contingency in these prelimbic lesioned animals. In a separate experiment, rats were again trained to lever press and pull a chain for food pellets and starch solutions respectively. Post instrumental training, half the animals received the food pellets when sated and starch when hungry. For these animals the food pellets should have low incentive value and starch high. The other animals experienced the reverse. In test the control group performed the action trained with the reward re-exposed in the hungry state more than the action trained with the reward re-exposed in the sated state. By contrast, animals with lesions to the insular cortex produced these actions a similar amount, suggesting a failure, in these animals, to code the incentive value of these rewards in various motivational states.

Berridge (2000) concluded that whilst the cognitive expectation model and Bindra-Toates incentive salience attribution usually work together to guide behaviour congruently, in some special occasions, such as a sudden shift in hedonic value without re-experience, a separation of the two can emerge.

Whilst under normal conditions, humans may not be able to distinctly tell 'wanting' and 'liking' apart (Robinson & Berridge, 1993). However, there are circumstances where they clearly diverge; for example, in drug addicts. The dopamine reward system, responsible for motivation to natural rewards such as food, sex, etc., is hijacked by drugs of abuse (Berridge & Robinson, 2003). These drugs change brain organisation, specifically those involved in incentive

motivation attribution. Neuro-adaptations make these systems hypersensitive to activating stimuli which, in turn, makes drug-associated stimuli profoundly salient and wanted.

1.11. Differences between Pavlovian and Discriminative stimuli

However, Robinson and Berridge's (1993) incentive salience theory merely addresses the psychological mechanism of learning as a unitary process without distinguishing between different types of learning. Specifically, the incentive salience theory commonly refers to Pavlovian stimuli which frequently signal *what* or *when* an outcome will occur. For example, to the dogs in Pavlov's experiments the metronome signalled *when* the food would be presented. However, most eating situations are embedded in some form of instrumental setting, in which an action needs to occur in order to obtain food. For example, whilst certain times of the day may not themselves be cues for food, they inform us whether a behaviour will be associated with food. Stimuli that signal *whether* an outcome will occur are called Discriminative Stimuli (DS). Importantly, discriminative stimuli appear to be distinct from R-S* and S-S*. Black, Osborne, and Ristow (1970) suggested that it might be best to conceptualise this as a S(R-S*) association, due to it being more than simply a synthesis of S-S* and R-S*. Research has shown that these discriminative stimuli perform differently from Pavlovian stimuli: Di Ciano and Everitt (2003) showed that whilst Pavlovian stimuli increased drug-seeking behaviour when presented contingent on responding, they had no effect when presented non-contingently. Conversely, DSs increased drug-seeking behaviour regardless of whether it was presented non-contingently or contingently. Similarly, rats with OFC lesions have been shown to have impaired performance on a differential outcome expectancy test (a measure of which action is produced in the presence of a DS), however show normal cue-potentiated feeding in the presence of a CS+ (McDannald, Saddoris, Gallagher, & Holland, 2005). Similar differences have also been found by Hovancik (1978). Additionally, Holman and Mackintosh (1981) have shown that conditioning to one element of a compound stimulus was blocked if that element was previously associated as a Pavlovian stimulus but not if it was a discriminative stimulus. In experiment two of that paper, a tone-light compound was trained as a discriminative stimulus for lever pressing. They found that the light stimulus suppressed responding, compared to control, when the tone was independently trained as a DS, but not if the tone was trained as a Pavlovian CS.

Empirically, studies investigating the effect of learning on ingestive behaviour has in certain areas (animal) been substantial, whilst in others (human) limited. Experiments investigating the effects of conditioning on appetitive behaviour have examined the effects of Pavlovian and

discriminative stimuli separately. Past research has typically investigated only one type of association, thus the following reviewed experimental research will be separated by the type of underlying learnt association.

1.12. Evidence for Pavlovian learning in animals and its effect on intake

Basic Pavlovian conditioning (stimulus-stimulus associations) is arguably the most extensively studied form of associative learning in vertebrates (Clark & Squire, 1998). It is relatively automatic and reflexive, and can occur even with extensive brain lesions to areas such as the hippocampus (Clark & Squire, 1998). For example, Weingarten (1983) showed that a conditioned stimulus could potentiate feeding behaviour. In his study a 4.5 minute CS+, consisting of a buzzer and light, were presented concurrently before every meal of a liquid diet based on evaporated milk. This highly palatable food was delivered in the last 30 seconds of the CS+. In comparison a CS- (an intermittent pure tone) was played at the midpoint of the inter-meal interval so was predictive of the non-availability of food. After repeated pairings of each the rats were sated and testing on their intake in the presence of each of the stimuli. Presentation of the CS+ increased intake, on average, by $20 \pm 2\%$ of their 24 hour intake. Interestingly, all the rats ate within five seconds of presentation of the CS+ which didn't happen in the presence of the CS-. However, the rats did compensate for this increased intake over 24 hours by reducing their food intake in their home cages.

If rats associate a specific context with food they have been shown to increase food intake in that context compared to rats who have never experienced the context with food (Calvin, Bicknell and Sperling, 1953a; Valle, 1968). The same effect was observed if rats had merely experienced the context in a state of deprivation (Calvin, Bicknell, & Sperling, 1953b). This cue-potentiated feeding has been shown to be specific to the cue-associated food and does not generalise to other familiar foods (Petrovich, Ross, Gallagher & Holland, 2007). This suggests that the CS acts in a specific and selective manner. However, there is evidence that the effect of a cue may be general rather than food-specific. In a study by Boggiano, Dorsey, Thomas and Murdaugh (2009), rats were conditioned to associate cages with distinct context cues (different bedding) with availability of highly palatable Oreo cookies. The critical finding was that rats consumed more chow when exposed to the context previously associated with the palatable food than a different context previously associated with only getting chow despite the chow not being the US previously associated with the CS+. This suggested that the CS+ elicited a general state of non-specific wanting of food which increased eating generally. Alternatively, since no Oreos were present during the potty training test, there was no opportunity for the rats to exhibit a cue-specific eating response. However, even if this is so,

the finding that chow-intake was increased at least demonstrates that cue-potentiated eating can generalise beyond the trained US.

1.13. Evidence for Pavlovian learning in humans and its effect on intake

However, whilst nonhuman animal models provide rigorously controlled investigations into cue-potentiated feeding, previous research (Rose & Corrigall, 1997) has shown differences in dopaminergic reward circuitry responses between humans and nonhuman animals. Whilst there are a large number of similarities in responses to appetitive rewards between humans and nonhuman animals (Rose & Corrigall, 1997), the noted differences suggest a separation of the two is vital. Additionally, psychological mechanisms that affect humans and not nonhuman animals may affect cue-potentiated feeding; for example, individual differences, such as dietary restraint and disinhibition, which have been shown to affect general dietary intake (Lawson et al., 1995).

In regards to humans, when a small prime (one bite of the reward) was the conditioned stimulus and pizza and ice cream were the reward, it was found that the prime stimulated intake compared to when no prime was eaten (Cornell, Rodin, & Weingarten, 1989). This increased intake was cue-specific (if participants were primed by pizza they ate more pizza than ice cream. Conversely, if they were primed with ice cream they ate more ice cream than pizza). This effect occurred whether participants were sated or not.

Odours can also serve as the conditioned stimulus. Jansen et al. (2003) showed that odours as well as a preload prime of the food itself can both increase subsequent intake, however this was dependent on weight status, with overweight children eating more after exposure to the odour cue and prime cue than normal weight individuals. Similar results were found by Jansen et al. (2008). Fedoroff, Polivy and Herman (1997) exposed low and high restraint (the tendency to restrict food intake to control body weight (Herman & Mack, 1975)) participants to either no cue (writing their thoughts generally), a cognitive cue (writing their thoughts on pizza), an olfactory cue (the smell of pizza), or a combination of the smell and cognitive cue.

Unrestrained eaters did not increase intake due to the smell or thoughts of pizza but restrained eaters did; specifically, thoughts and smell of pizza increased intake and craving ratings compared to free thoughts or no smell respectively. Also, Fedoroff, Polivy and Herman (2003) divided participants to one of three conditions; either participants were exposed to a pizza cue, a cookie cue, or no cue. Specifically, whilst participants wrote their thoughts about pizza, cookies or in general (depending on the condition) one of the corresponding odours (no odour in the control condition) was emitted into the room. They were then given a plate full of either pizza or cookies and ate as much as they wanted for 10 minutes. After this they received

the other plate of food. Fedoroff et al. found that intake was greater in the cued conditions than the no-cue condition. When subdivided by restraint Fedoroff et al. found that restrained eaters showed cue specificity; eating more only when they had been previously cued with that specific food (increase in pizza intake when cued by the pizza odour but not the cookie odour). If participants had not been cued with the food odour before it was made available to them, they actually ate less than unrestrained eaters. Interestingly, both unrestrained and restrained eaters reported increased appetite for pizza when cued but only restrained eaters went on to eat more pizza. However, the empirical findings of cue-potentiated feeding elicited by food-associated odours is far from consistent. A number of studies have failed to find odour-potentiated feeding at all (Zoon, He, de Wijk, de Graaf & Boesveldt, 2014; Nederkoorn & Jansen, 2002), whilst others have found contradictory findings (Jansen & Van den Hout, 1991). The sight of food can stimulate intake. Marcelino, Adam, Couronne, Köster and Sieffermann (2001) showed participants either a picture of pizza or a variation of the visual quality of the food stimulus. They found that appetite for pizza increased after exposure to the food stimulus, and this reported appetite was a predictor of the amount of pizza subsequently eaten. This has been shown to be dependent on weight status (Nisbett, 1968), and restraint (Coelho, Jansen, Roefs, & Nederkoorn, 2009; Rogers & Hill, 1989). However, again, the consistency of this finding has been disputed (Lambert, Neal, Noyes, Parker, & Worrel, 1991). The aforementioned research on Pavlovian conditioning has provided strong evidence for cue-potentiated feeding in humans and nonhuman animals. These conditioned stimuli appear to act in a specific manner, although there is noted research to the contrary. What is notable is the large array of stimuli shown to increase eating such as a food-associated context, a prime of the food itself, odours and images of the food. Another important finding has been the role of individual differences, such as restraint, in influencing cue-potentiated feeding. It is thus critical to control for these factors in future human research in this area.

Research has provided evidence that conditioned stimuli can modulate ingestive behaviour. In particular, two theories of associative learning have attracted interest; flavour-nutrient learning (FNL) and flavour-flavour learning (FFL). FNL suggests that liking or disliking for a flavour reflects associations between the flavour (which is a conditioned stimulus) and the post-ingestive effects (the unconditioned stimulus). FFL suggests that through repeated pairing of a novel flavour (CS) with an already liked or disliked flavour (the US) modulates the liking for the flavour CS (Yeomans, Leitch, Gould & Mobini, 2008). These forms of evaluative conditioning (a change in liking of a stimulus that result from pairing that stimulus with a positive or negative stimulus [De Houwer, Thomas, Baeyens, 2001]) have been regarded as a form of Pavlovian conditioning (De Houwer et al., 2001).

Whilst the evidence for FNL in humans is fairly strong (Sclafani, 1999; Booth, 1972; Capaldi, 1992), the evidence in human studies is weaker, with the reliability of findings weaker (see Brunstrom, 2005). One issue according to Yeomans et al. (2008) is the co-occurrence of FFL and FNL in experimental studies. For example, the taste and palatability of a US can be confounded with post-ingestive properties. Future studies need to isolate these components by, for example, only investigating the effects of a palatability on conditioning behaviour.

Dietary restraint has also been shown to affect FNL (Yeomans, 2012). For example, women who score high on the restraint scale show insensitivity to the manipulated energy levels of food which may suggest insensitivity to internal cues (Brunstrom & Mitchell, 2007). Brunstrom, Downes, and Higgs (2001) showed that restrained eaters are, also, less likely to acquire or express flavour-flavour associations. Similar to Schachter's (1971) eternality theory of obesity, these studies would suggest an insensitivity to internal cues and, potentially, overly sensitive to external cues. Jansen and Van Den Hout (1991) observed dieters overate after smelling a preload, while dieters without this preload could maintain intake control. Jansen (1990) hypothesized that this is conditioned craving elicited by the preload.

One interesting observation by Baeyens, Eelen, Van Den Bergh and Crombez (1990) was that contingency awareness may not be necessary for evaluative conditioning to occur. When colours were used as the CSs no differences between aware and unaware participants emerged, however, when flavours were used as the CSs no participants were aware and yet evaluative changes occurred. This may suggest a distinction between external and/or visual stimuli and internal and/or gustatory stimuli.

1.14. Problems with previous Pavlovian learning studies and their effect on intake

The animal research commonly takes an arbitrary stimulus and trains animals to associate this initially neutral stimulus with receiving a US, commonly food. Human research has frequently taken a US and investigated the conditioned stimuli that are commonly associated with it; an almost reverse of the animal experimental philosophy. Whilst the human research is interesting and has enhanced our understanding of cue-potentiated feeding in humans, a number of issues with the aforementioned research remain. For example, it is well known that the number of CS-US pairings affects not only the amount of learning, but also what is learnt and the consequences for behaviour (Baeyens, Eelen, Crombez, & Ven den Bergh, 1992). For instance, Valle (1968) showed that the more amount of time a rat experienced a CS-US relationship, the more they consumed in the presence of the CS. Additionally, it is now well established that more extensive experience with CS-US pairings (for example, overtraining) can alter the associative structure underlying Pavlovian memories, as well as the neural basis of

these memories (Rabinak & Maren, 2008). For example, lesions to the basolateral amygdala disrupts conditioned fear response acquisition with limited training, but does not disrupt this acquisition with overtraining (Maren, 1998, 1999). The average 21 year old will have consumed over 23,000 meals in their lifetime which suggests the majority of normal dietary behaviour is overtrained (Brunstrom, 2007). Thus, not being able to determine or control the number of CS-US pairings in human studies by using more naturalistic stimuli may result in legitimate difficulties when interpreting the results of such studies.

Also, Tetley, Brunstrom and Griffiths (2009) highlighted that typically in human cue-potentiated eating studies, intake in a control condition is compared to that in a food-cued condition. However, if the presentation of food immediately prior to eating acts as a cue which stimulates intake (Weingarten, 1985), then the non-cued condition becomes a cued condition. Additionally, individual differences have been both methodologically and statistically ignored in previous cue-potential studies. For example, restrained eaters shown both salivary (Brunstrom, Yates, & Witcomb, 2004) and ingestive (Jansen & van den Hout, 1991) differences in response to food cue exposure. Additionally, restrained eaters have different affective value of stimuli compared to unrestrained eaters. In a flavour-flavour learning experiment (in which repeated compound-presentations of a novel flavour (CS) with a liked or disliked flavour (US), causes the affective value of the novel flavour to change in the direction of the US), unrestrained eaters show liking changes to the CS flavour in a dose-dependent manner when the unconditioned stimuli are 10%, 50% and 90% sucrose solutions (Brunstrom, Higgs, & Mitchell, 2005). Restrained eaters, however, only show liking changes to the CS paired with the 10% sucrose solution. This suggests that the same unconditioned stimuli can have markedly different hedonic values dependent on the individual's cognitive beliefs about that stimulus. In previous research, individual differences such as restraint, and hunger (which has been shown to also affect conditioning (Yeomans, 2006)) have not been controlled for.

To control all the factors just mentioned, an experiment using human participants must use an initially neutral and novel stimulus and control the contingency between the CS-US.

1.15. Evidence for Pavlovian learning in humans and its effect on intake

To date only one study in humans has used an explicit cue-food training paradigm to explore cue-potentiated eating in humans (Birch, McPhee, Sullivan, & Johnson, 1989). In their experiment preschool children (mean age of 39 months) were conditioned to associate one stimulus (a rotating red light, placed on the same table as to where the food was presented, and a piece of music presented 30 seconds before the food appeared and continuing for four and a half minutes) with the availability of palatable snack foods. During the presentation of

this CS+ children could do a range of activities, such as building things and listening to stories, whilst eating as much as they wanted for the 20 minute eating period. On different days a CS- stimulus (a red, yellow and green traffic light and a different piece of music) was presented with no food present. After the two pairs of test trials (one CS+, one CS-), children were asked if they were aware of the associations between the cues and whether food was presented or not (four of seven were aware). In the presence of the CS+ children ate 75 kcal more than on the CS- but only if they were aware of the association; those unaware did not eat differently in response to the different stimuli. In a second experiment (all children were aware of the contingency between the stimuli and whether food was presented or not), Birch et al. again showed that in response to the presentation of the CS+ stimuli children ate significantly more (190 kcal) in the presence of the CS+ than the CS- (118 kcal). Interestingly, all 15 children initiated eating within one second after CS+ presentation. In comparison on CS- presentation only 3 out of the 15 children ate within one second. Whilst Birch et al (1989) interpret their finding as an increase in intake in the CS+ group, their difference in consumption may be due to a suppression of intake in the CS-.

1.16. Evidence for Pavlovian learning in humans and its effect on physiology

Whilst evidence for cue-potentiation of intake is slightly contradictory, the evidence for the effect of conditioned stimuli on appetitive physiological responses is more straightforward. For example, the sight and smell of food have separately been shown to elicit changes in physiology such as increased heart rate, gastric activity (Nederkoorn & Jansen, 2002), and saliva production (Rogers & Hill, 1989), as well as increasing attentional bias towards these stimuli (Castellanos et al., 2009). Together, this compound stimulus has also been shown to increase heart rate, diastolic and systolic blood pressure, gastric activity, temperature, skin conductance, and swallowing rate (Nederkoorn, Smulders, & Jansen, 2000; Epstein, Truesdale, Wojcik, Paluch, & Raynor, 2003). As with intake, this has been shown to potentially be dependent on restraint status (Klajner, Herman, Polivy & Chhabra, 1981; Sahakain, Lean, Robbins, & James, 1981). Klajner et al. (1981) had participants smell freshly baked pizza before salivatory response was measured. Whilst they found that dieters salivated more than non-dieters, they also found that this was dependent on the palatability of the stimulus; when the study was repeated with an unpalatable food stimulus (a green chocolate chip cookies) the salivary differences between dieters and non-dieters was eliminated. This suggests that the reward value determined the physiological response to the stimulus associated with it. These studies suggest that conditioned stimuli produce cephalic phase responses to prepare the

individual for consumption by optimizing the body for digestion of this food. However, this physiological reactivity does not always lead to subsequent ingestion (Nederkoorn et al., 2000). In regards to initially novel and neutral stimuli, Van Gucht, Vansteenwegen, Beckers and Van den Bergh (2008a) trained participants to associate a particular tray with the delivery of chocolate. Participants were presented with one of two trays (one rectangular and white, and another round and green). They were told that one of the trays would sometimes be followed by the opportunity to taste chocolate, whereas the other would not. Initially participants were presented with one of the trays while rating their craving and expectancy to receive chocolate. Then a piece of chocolate in aluminium foil was placed on the tray and participants were instructed to unwrap it and smell the chocolate intensely for one minute, before rating their craving for the chocolate. Then depending on the tray the participant was either allowed (CS+) or not allowed (CS-) to consume the chocolate. After conditioning, participants experienced extinction either in the same context as the conditioning took place or a novel context, before test in the same context as conditioning took place. Interestingly, they suggested that they found that US-expectancy showed acquisition, extinction and contextual renewal (greater expectancy in the group that extinguished in the novel context) but craving did not. These findings were replicated in a subsequent study (Van Gucht, Vansteenwegen, Van den Bergh, & Beckers, 2008b) which also added a stimulus-response compatibility (SRC) task. In this trial a manikin figure was displayed and participants instructed to either move the manikin toward pictures of the serving tray that had been associated with chocolate (CS+) or away from the tray not associated with chocolate (CS-). Instructions were reversed (i.e. away from CS+, towards CS-) for another block of trials. The US-expectancy replicated what was previously shown in Van Gucht Vansteenwegen, Beckers and Van den Bergh (2008a), however only in the group that extinguished in a novel context. The results of the SRC task also showed a significant effect only for the group that extinguished in a novel context; they were significantly quicker to approach the CS+ or move away from the CS-, compared to moving away from the CS+ or approaching the CS-. In the group that extinguished in the same context as conditioning took place no approach tendency was observed. a slight contradictory finding in the second experiment of this paper (the same SRC effect but no contextual effect) may be due to the replacement of the aluminium foil with the chocolate's original wrapping. These studies highlight the contextual specificity of conditioned stimuli; if the context informs an individual that the stimulus-food association is no longer valid then the individual does not expect the reward. In other words, a S-S* association is contextually specific.

1.17. Evidence for instrumental conditioning and the effects of discriminative stimuli on animals

As initially mentioned in the present chapter, Thorndike (1898) showed that cats will produce a response in order to escape a box. Since this study there have been copious R-S* learning studies, such as thirsty rats will lever-press for the delivery of water (Hammond, 1980).

Similarly, Dickinson & Mulatero (1989) trained rats to respond on two levers, each giving access to a different reward. The introduction of free outcomes of a given type resulted in a decline in responding that was particularly marked on the lever that was associated with that food-type. Additionally, Dickinson, Campos, Varga and Balleine (1996) trained rats to push a single manipulandum, a rod, in one direction for a food pellet and another direction for starch. When one of these was devalued (through pre-feeding of one of the reinforcers before test) rats responded selectively by responding less to the newly devalued reinforcer. This type of responding has been shown to be dependent on reinforcer value (Crespi, 1942; Adams & Dickinson, 1981; Colwill & Rescorla, 1985a).

However, responding for food in the real world will seldom occur in isolation. Specifically, there are often cues that inform us *whether* a specific behaviour is likely to be rewarded (discriminative stimuli). Instrumental performance can be conditioned to these discriminative or contextual stimuli through Pavlovian conditioning (Dickinson & Balleine, 1994; Bouton, Rosengard, Achenbach, Peck, and Brooks, 1993). Detke, Brandon, Weingarten, Rodin and Wagner (1989) trained rats to associate food to a 10 second visual cue only when one 35 second tone was played but not another. These tones are context-like, according to the researchers, as it informs the subjects whether the CS visual cue predicts the reinforcer. When both the tone and visual cue were present rats showed greater conditioned responding (headpoking) compared to when a tone which wasn't associated with food and the visual cue was presented or when the visual cue was presented alone (though this is not a consumption experiment). The CS+ also increased food magazine approach behaviour as well as insulin release. Crombag and Shaham (2002) trained rats to associate lever pressing with delivery of a mixture of heroin and cocaine. After this training they either received extinction sessions in the original training context or in a different context. After this extinction training rats were returned to the original drug-associated context. The rats that had been extinguished in a different context showed powerful renewal, which wasn't shown in the rats who had extinguished in the same context. Similar results have been shown with shock training (Bouton & Bolles, 1979). A variety of stimuli can act as discriminative stimuli: environmental context (Bouton, 1993), drug state (Bouton, Kenney, & Rosengard, 1990), time (Bouton, 1993), and internal deprivation state (Davidson, Flynn, & Jarrard, 1992). Discriminative stimuli have also

been shown to be like Pavlovian stimuli in the sense that they are highly response-specific, and do not modulate other responses evoked by other excitatory stimuli (Holland, 1986). Critically, these discriminative/contextual stimuli modulate R-S* associations whilst having no direct S-S* association themselves (Bouton et al., 1993; Bouton & Swartzentruber, 1986).

1.18. *Evidence for the effects of discriminative stimuli on humans*

A range of behaviours have been shown to be able to be under control of instrumental conditioning, such as vasodilation (Razran, 1961), heart beats (Brener, Kleinman & Goesling, 1969), salivation (Brown & Katz, 1967), and many others (see Kimmel, 1974). In regards to control of these behaviours by discriminative stimuli however, whilst there is substantial evidence for the effect of discriminative stimuli in humans in a range of areas, such as cannabis (Chait et al., 1988), investigations into the effects of discriminative stimuli on human ingestive behaviour is more limited. To investigate satiety, Havermans, Janssen, Giesen, Roefs, and Jansen (2009) allowed participants to consume 250ml of chocolate milk prior to pressing a button for points they could trade for a certain reward (either earning points for crisps (one group) or chocolate (another group)). They observed a food-specific decrease in both wanting (measured by number of presses on a progressive ratio schedule) and liking (measured by explicit subjective visual analogue scale (VAS) ratings) of chocolate compared to crisps. However, there is no study that I am aware of that has investigated the effect of discriminative stimuli on intake in humans.

1.19. *Liking and wanting in humans*

Robinson and Berridge's (1993) incentive salience theory postulates that reward is made up of three distinct parts; associative learning, pleasure and incentive salience ('wanting'). Whilst there has been a large amount of research investigating associative learning and intake, there has been limited investigation of 'wanting' and 'liking' in regards to intake in humans, with a few notable exceptions. First of note, chronologically, would be the work of Leonard Epstein. Epstein developed the Relative Reinforcing Value (RRV^{food}) of food task (Raynor & Epstein, 2003). In this task points are earned for either food or a sedentary activity. Participants pressed a button of a joystick in order to obtain points for their chosen reward: the number of presses needed to obtain this reward increased across trials based on a progressive ratio schedule. Epstein suggested that the more one was willing to work for a reward denoted how much that person 'wanted' that reward. The RRV^{food} task has shown that food was more reinforcing for a deprived condition compared to a fed condition, with no differences observed for the subjective or objective (measured by hedonic facial reactions) hedonic measures

(Epstein et al., 2003). A criticism of this work was that the hedonic reactions were conducted on foods (chocolate milk, lemon juice and water) different from those used in the RRV^{food} task (snack foods), however this was addressed by Raynor and Epstein (2003) who found similar results. The RRV^{food} task has revealed differences in the reinforcing value of food for obese compared to normal-weight individuals (Saelens & Epstein, 1996; Temple et al., 2009); individual genetic variations (Epstein et al., 2004b); and an interaction between the two (Epstein et al., 2007). It has, also, been shown to be a good predictor of food intake, both in an *ad libitum* taste test and usual daily energy intake (Epstein, Carr, Linn, & Fletcher, 2011; Epstein et al., 2004a). Interestingly, whilst scores on the RRV^{food} task are a good predictor of intake, hedonic measures are unrelated to intake (Epstein et al., 2004a; Epstein et al., 2007; Epstein et al., 2011).

Another task that has been suggested to measure both 'liking' and 'wanting' is the Leeds Food Preference Questionnaire (LFPQ) developed by Graham Finlayson and John Blundell. In this task there are two sections; firstly, VAS ratings of both explicit wanting ('how much do you want some of this food now?') and explicit liking ('how pleasant would it be to taste this food now?') for individual foods are taken. In a subsequent task, two food pictures are presented on screen and participants asked 'which food do you most want to eat now?'. The food selected and reaction time to selection provide a score of implicit 'wanting'. The LFPQ has shown significant differences in hungry compared to satiated participants (Finlayson, King, & Blundell, 2007a); hungry participants wanting high-fat savoury foods more than low-fat savoury foods but with no differences in explicit liking, and liked high-fat sweet foods more than low-fat sweet foods but no differences in 'wanting'. In comparison, satiated individuals liked but did not want high-fat savoury foods more than low fat savoury foods and wanted but did not like low-fat sweet foods compared to high-fat sweet foods. It has, also, been shown to be sensitive to differences post-exercise (Finlayson et al., 2010), post-meal (Griffioen-Roose, Finlayson, Mars, Blundell, & de Graaf, 2010; Finlayson, Arlotti, Dalton, King, & Blundell, 2011; Cameron, Goldfield, Finlayson, Blundell, & Doucet, 2014; Finlayson, King, & Blundell, 2008); and due to individual eating traits, such as disinhibition (Finlayson, Bordes, Griffioen-Roose, de Graaf, & Blundell, 2012), binge-eating (Dalton, Blundell, & Finlayson, 2013a; Dalton, Blundell, & Finlayson, 2013b) and anorexia nervosa (Cowdrey, Finlayson, & Park, 2013). It has been shown to be a good predictor of food choice and intake (Griffioen-Roose et al., 2011), and better at predicting daily energy intake than the RRV^{food} task (French, Mitchell, Finlayson, Blundell, Jeffery, 2014).

According to the RRV^{food} task it is wanting that drives intake rather than the hedonic value of the food. However, research using the LFPQ has suggested that wanting generally to all foods might be misleading; separating food into categories, such as high-fat sweet, low-fat sweet, etc., provides more detailed ‘wanting’ and ‘liking’ differences, which may depend on weight status, individual eating traits, and hunger status. However, neither of these tasks have been used to investigate the psychological mechanisms behind cue-potentiated feeding using initially novel, visual stimuli.

1.20. Future directions

Whilst there is phenomenological evidence for cue-potentiated feeding in humans, the majority of research has been atheoretical; merely investigating the effect of the cue on intake. We have substantial evidence for the effect of naturalistic cues, such as odours, on intake, however, with the exception of the study by Birch et al. (1989), what remains to be investigated is the effect of arbitrary and initially novel cues on intake, in particular in adults (Birch’s paper was conducted with children). Whilst it is to be acknowledged that by using arbitrary and initially novel cues one loses translational applicability to a real world setting, it does mean you can investigate phenomena that would otherwise be unobtainable using naturalistic stimuli, such as odours. For example, reward uncertainty which can influence the incentive motivational power of CSs (Anselme, Robinson, & Berridge, 2013), the effect of which has not been investigated in human cue-potentiated feeding.

The key issue with the majority of research into cue-potentiated feeding is the lack of control of the stimulus-reward association. The majority of previous research have investigated previously established stimulus-reward associations and their effect on intake. As previously mentioned, not knowing and controlling the number of CS-US associations assumes equality when considerable individual variation may exist. This could explain the contradictory findings in various experiments. Rigorous control of the formulation of these associations, as well as participants’ explicit awareness of them which has been largely ignored, will allow strict and accurate conclusions to be drawn.

It, also, remains unknown which aspect of reward is driving behaviour in cue-potentiated feeding in humans. Measures of liking and wanting have yet to be investigated in regards to cue-potentiated feeding. Studies that have investigated behavioural responses to initially novel food cues in humans have mainly used subjective ratings which, although may give an indication of behaviour as suggested by other research (Nederkoorn and Jansen, 2002; Lambert et al., 1991), may not represent actual food intake (Zoon, He, de Wijk, de Graff, & Boesveldt, 2014). However, subtle measures of wanting and liking, such as the LFPQ, may offer

a more accurate method of measuring these psychological processes, implicit wanting in particular, which cannot be measured by simple subjective measures.

It is, also, not known what aspect of reward is needed for cue-potentiated feeding. In previous research it is not clear what exactly the cues are predicting. By this we mean, are these cues associated with the receipt of calories? Or palatability? The availability of food? Or a number of these food-related aspects simultaneously? Using complex USs, as in the case of food such as pizza, negates to answer the important question of what specific aspect of the US leads to cue-potentiated feeding. Future studies should look to address this by dissecting the US.

1.21. Summary and aims

Research has shown that Pavlovian cues associated with food increase subsequent consumption in the presence of the conditioned stimulus. Discriminative stimuli have also been shown to increase appetitive behaviour. However, to our knowledge there has been no investigation into the effects of initially novel and arbitrary stimuli associated with food on consumption in human adults.

The experimental work in this thesis will be divided into three chapters, each investigating distinct research questions. Chapter One will investigate whether Pavlovian stimuli associated with a sweet taste can increase subsequent snack intake in the presence of this cue compared to a stimulus associated with a neutral taste. It will also investigate the specificity of the cue by asking whether this increase will be solely of sweet foods or to foods in general. Chapter Two will investigate whether discriminative stimuli produced the same behavioural effects as Pavlovian stimuli, and whether these phenomena occur when sated and non-sated. Lastly, Chapter Three will investigate whether Pavlovian and discriminative food-associated stimuli are represented differentially in the brain.

The aims of this thesis are to investigate how food-associated Pavlovian and discriminative stimuli affect subsequent intake, in addition to looking at how they are represented in the brain. Each chapter will contain an introduction into the research area, at the end of which specific aims will be outlined.

Chapter 2: Conditioned sweet-paired stimuli elicit potentiated feeding in humans.

2.1. Introduction

The number of people who are overweight or obese has doubled since 1980 (World Health Organisation, 2011), and this rise is attributed to a positive energy balance where energy intake exceeds expenditure (Levine, 2005). The multifaceted nature of eating (e.g. Birch & Fisher, 1998) makes it difficult to identify the cause of the overeating underlying this imbalance in energy. Whilst biological causes have been extensively investigated (Havel, 2001; Woods, 2005; Woods, Seeley, Porte, & Schwartz, 1998), the role of external influences on eating has been investigated to a lesser degree, and in particular the potential role of learning in overeating remains unclear.

One idea is that environmental stimuli (cues such as visual stimuli) which are associated with food through Pavlovian conditioning come to elicit conditioned responses (CRs) which involve increased intake. This has been shown to be the case in non-human animal models: in sated rats, initially arbitrary and novel cues that are consistently paired with the availability of food have been shown to enhance intake in sated rats (Grant & Milgram, 1973; Galarce, Crombag & Holland, 2007; Holland & Gallagher, 2003; Galarce & Holland, 2009; Valle, 1968; Detke, Brandon, Weingarten, Rodin, & Wagner, 1989). For example, Weingarten (1983) compared the effect of a conditioned stimulus (CS) presented immediately before food availability to a cue presented at the midpoint of every intermeal interval, on rats feeding behaviour. He found that even when sated, the food-associated cue instigated intake with a mean latency of less than five seconds and increased short-term energy intake by approximately 20% compared to the between-meal cue. Similarly, Zamble (1973) demonstrated that a light or an auditory signal indicating a feeding period caused a 10-15% increase in feeding in rats compared to stimuli non-predictive of food presentation. Thus, cues that predict the onset of food availability (meal cues) reliably increase short-term intake in rats, although notably these cues did not result in increase in total daily energy intake even when a cued meal constituted as much as 50% of total daily intake (Weingarten, 1983). The ability of cues to enhance short-term intake has since been termed cue-potentiated eating, and these early studies replicated and extended (Petrovich, Ross, Gallagher, & Holland, 2007).

While cue-potentiated feeding is well characterized in non-human animal models, detailed experimental analysis in humans is lacking. In one of the few human conditioning studies using

a novel CS-food pairing, Birch, McPhee, Sullivan, and Johnson (1989) gave preschool children snack food in the presence of one stimulus (CS+) and toys in the presence of another (CS-). They found an important distinction: those children aware of the association between the Conditioned Stimuli and the snack foods ate significantly more (75 calories more) in the presence of the CS+ compared to when the CS- was presented. Those unaware of the association did not eat significantly differently in the presence of the CS+ or CS-. They also found in the presence of the CS+ the latency to first food choice of the aware children was significantly faster (all 15 children ate with a latency of less than one second) than in the CS- group.

However, it remains unknown what specific aspect of food the CS is cueing the enhanced eating in Birch et al.'s (1989) experiment. It could have been the hedonic quality of the snack foods that were associated with the novel stimuli or merely food availability, as in the previously mentioned non-human animal research. Additionally, in the human cue-potentiated feeding literature the cues associated with food were not specific to any individual food item, but were more general cues predicting the likely availability of food (Birch et al., 1989), and so it was unlikely that any sensory-specific associations were established. Thus, it remains unknown whether food-associated cues elicit specific or general increased intake.

The present study explored further cue-potentiated feeding in adults based on associations between novel visual cues and a sweet taste to test for the first time whether a visual cue associated with a hedonic reward modified subsequent eating. Initially, participants who were sweet-likers (Looy & Weingarten, 1991) were exposed to multiple pairings between a novel visual stimulus and different tastes using a disguised training method, in which participants were told to identify an odd solution from three solutions (Stevenson, Prescott, & Boakes, 1995; Yeomans, Mobini, Elliman, Walker, & Stevenson, 2006). Post training participants were given free-access to sweet and savoury snacks labelled with either the cue paired with the sweet taste or a cue paired with a neutral taste. By offering conditioned stimulus consistent and conditioned stimulus inconsistent food, this study investigated whether the conditioned stimulus elicited a representation of its paired outcome (cue specific consumption) compared to a motivational state (Petrovich, Ross, Gallagher, & Holland, 2007). Contingency awareness was also measured.

It was predicted that when the cue that had been experienced with a sweet taste (CS_{sweet}) was present in the free feeding situation participants would eat more overall, and in particular more of the sweet flavoured items, compared to those participants presented with the same snacks labelled with the cue associated with a neutral taste (CS_{neutral}).

Experiment 1

2.2. Method

Table 2.1. Method synopsis.

	Method	Task
Day 1	<i>Taste screening session</i>	Participants tasted 10% sucrose solution on pleasantness and sweetness to ascertain if they were 'sweet-likers'.
Day 2	<i>Control of hunger state</i>	Standardised lunch.
	<i>Conditioning phase</i>	Participants associated stimuli on cups with tastes within.
	<i>Contingency awareness test</i>	Presentation of stimuli and asked which tastes were associated with each stimulus.
	<i>Intake test</i>	Participants were presented with a selection of food items with the conditioned stimuli on the bowls containing the food/drink.

2.2.1. Design

A between subjects design was used to compare the effects of a novel visual cue that had been paired with the taste of 10% sucrose solution (CS_{sweet}) and a second cue that had been paired with a neutral taste (CS_{neutral}) on subsequent intake of sweet and savoury foods and drinks in a taste evaluation task.

2.2.2. Participants

Fifty healthy males ($n = 22$) and females ($n = 28$) initially consented to participate in a study described as involving "taste perception". Data from five of these participants were excluded due to not following experimenter instructions, and computer error. The 45 remaining participants had a mean age of 22 (range 18-35) and BMI of 23.3 ± 0.40 kg/m².

The experimental protocol was approved by The University of Sussex's Ethics Committee and conformed with British Psychological Society rules on ethical conduct. Participants gave written informed consent at the start of the test session, and they were compensated by payment of £7 and/or awarded course credits on completion of the study.

Because a history of dieting may decrease sensitivity in flavour-based learning (Brunstrom, Downes, & Higgs, 2001) and alter responses to food cues (relative to unrestrained eaters) (Fedoroff, Polivy & Herman, 1997), participants first completed the Three Factor Eating

Questionnaire (Stunkard & Messick, 1985): participants scoring seven or higher on the Restraint scale were not invited to take part in the study. Participants with a history of eating disorders, who were diabetic, had specific allergies to any ingredients used in the study, who smoked more than five cigarettes per week or who presently used prescription medication (excluding the contraceptive pill) were excluded.

2.2.3. Taste Screening Session

The key association in this study was between a novel visual cue and a liked sweet taste (10% sucrose solution). Past research shows individual differences in 'sweet-liking' (Looy & Weingarten, 1992), may influence performance in sucrose-based associative learning (Yeomans et al., 2006, Yeomans, Prescott, & Gould, 2009). To distinguish sweet-likers from sweet-dislikers, participants attended a short test session at least one day before the main test and rated two samples of 10% sucrose solution and two samples of water for pleasantness, sourness, sweetness, bitterness and saltiness using visual analogue scales, end-anchored with 'Very pleasant' (scored 100) and 'Very unpleasant' (scored 0) for rating flavour pleasantness and 'Extremely' (scored 100) and 'Not at all' (scored 0) for the other ratings. The label for the dimension evaluated was written above the centre of each line. Ratings were made using Sussex Ingestive Pattern Monitoring (SIPM, version 2.013, University of Sussex). Ten millilitre samples of each solution were refrigerated and brought to room temperature prior to tasting. Participants were defined as sweet-likers if their average rating of the two sucrose solutions was at least 55pt on both the pleasantness and sweetness scale. This procedure was based on previous studies of sweet liking (Yeomans et al., 2009; Looy & Weingarten, 1992).

2.2.4. Stimuli

2.2.4.1. Conditioned stimuli. The visual cues were adapted from a series of images constructed by Kuwayama (1973) and previously used in cue-based food learning (Laane, Aru, & Dickinson, 2010). To select images that were distinct and neutral in terms of liking, a pilot study was conducted where 12 participants rated the novelty and hedonic appeal of 29 images. Five images rated as high in novelty and neutral in liking were selected as stimuli for use in this study (see Figure 2.1). Stimuli were counterbalanced across participants.

2.2.4.2. Unconditioned stimuli. There were five sets of unconditioned stimuli, each with three 150ml liquid samples presented in 200ml cups. Within sets A and B the solutions in each sample were the same. In set A (the sweet set) the solutions were 10% sucrose solution. In set B (the neutral set) the solutions were "artificial saliva" [which consisted of deionised water, 1.865g/l of potassium chloride (KCl), and 0.210g/l of sodium bicarbonate (NaHCO₃) as used by

Zampini, Sanabria, Phillips, & Spence (2007)]. In set C (additional set) two solutions were weakly Açai (10 drops per 250g water [Givaudan©]) and one was strongly Açai (35 drops per 250g water [Givaudan©]). In set D (additional set) two solutions tasted of Grapefruit (30 drops per 250g water [Givaudan©]) and one was water. In set E (additional set) one solution tasted of raisin (30 drops per 250g water [Givaudan©]) and two were water.



Figure 2.1. The five abstract stimuli used in the conditioning task.

2.2.5. Control of hunger state

Hunger can alter brain activation (LaBar et al., 2001), in areas associated with stimulus salience attribution (Amaral, Price, Pitkänen, & Carmichael, 1992). For this reason participants were given a 554kcal standard lunch which consisted of a cheese and cucumber sandwich (40g of cheddar cheese, 30g of cucumber and two slices of white bread [Kingsmill Great Every Day]), a 34.5g packet of crisps (ready salted, Walkers brand), five cherry tomatoes and a glass of water.

2.2.6. Procedure

2.2.6.1. Standard lunch. Participants who had passed the sweet-liker assessment arrived at the laboratory between 1200-1400 for the start of the test day, and consumed the standard lunch. They then were allowed to leave the laboratory for two hours but were instructed to refrain from eating and to only consume water during this time. On returning participants indicated their hunger and thirst on separate 100pt line scales from 0 (not at all hunger/ thirst) to 100 (extremely hungry/thirsty) disguised as a mood rating questionnaire, presented using SIPM. These questions were integrated into a larger mood questionnaire measuring 'How energetic/calm/clearheaded/strong is your desire to eat/much do you think you could eat/full/tired/headachy do you feel right now?' on separate 100pt scales from 0 (not all all) to 100 (extremely). All testing was conducted in small air-conditioned cubicles at the University of Sussex.

2.2.6.2. Conditioning phase. The conditioning phase used the triangle-test disguised training method (Stevenson, Prescott, & Boakes, 1995; Yeomans et al., 2006). There were five sessions in total, with five trials in each session (one presentation of set A, B, C, D and E). During each trial participants were presented with three solutions. Using E-Prime (version 1.2) participants were instructed the following:

“For this task, you will be presented with an odd-one-out task. You will be asked to take one of the Sets of drinks and place it in front of you. Your task will be to taste each solution and determine which solution is the odd-one-out based on its taste. Some trials will be hard and some will be easy. You must select the solution that you think is the odd-one-out by its location on the tray. For example, if you think the solution on the left of the tray is the odd-one-out then click the picture on the left of the screen. When you try a solution put it back on the tray. Do not pick up the next solution until you have returned the first solution to the tray. Spit the solution into the bottle provided then swill your mouth with water. Please only take one sip of each solution per task.”

The would-be conditioned stimuli associated with each set were presented in two locations; first using 4 x 4 cm stickers directly placed on the cups and secondly on a 17” LED screen located on a desk in front of the participant (the three images presented equidistance along the screen with the question centred above them). The three cups in each set had the same CS sticker on the side of it which was specific to the solution in the cup (i.e. one CS for sweet solutions, one CS for neutral solutions, etc.). All three CS’ on screen and the cups were the same image. Participants were choosing which CS to pick based on its location corresponding to the samples on the tray. Participants tasted the three samples and once they identified an odd sample had to click on one of the three CS’ on the screen.

To ensure participants understood the task a practice trial (one solution orange and mango squash and two water) was conducted with the experimenter present. For sets A and B the three solutions were identical. This forced participants to closely attend to the flavour of the solutions and also to the stimuli presented on the screen. Sets C, D and E were control stimuli, used to disguise the purpose of the study. Participants were instructed to only take one sip of the solutions per session as this would ensure a standard 25 CS-US pairings for all participants. After each US sampling, participants were instructed to spit the solution and rinse their mouth with distilled water. Once participants had completed all five trials participants completed four more sessions (total of 25 trials).

All images, tastes and order of tasting was counterbalanced and randomised across participants.

2.2.6.3. Contingency Awareness. After completing the conditioning task, participants completed a contingency awareness questionnaire in which participants were presented with every CS and asked 'If you saw the following picture, what taste would you expect to experience?'. They were then given five tick box options (sweet, bitter, neutral, unsure and salty) and had to select one. They were then instructed to wait in an adjacent room for 30 minutes while the testing room was prepared for the cue-potentiated intake test.

2.2.6.4. Intake test. At the start of the intake test, participants were informed that they would now complete a taste evaluation task. Participants were informed using SIPM that prior to conducting a series of VAS ratings of foods and drinks over a five minute period, that during this period they must try each food and drink at least once, but that they could eat and drink as much as they wanted as any remaining food would have to be discarded. The experimenter then provided participants with a tray containing four bowls (two with sweet foods and two with savoury foods [see Table 2.2]), one glass of sweet drink and one glass of neutral drink) on a tray. For participants in the CS+ condition, the outside of the bowls and glasses contained the CS_{sweet} stimulus sticker, while in the CS- condition the bowls and glasses contained the CS_{neutral} cue on the outside. Participants clicked an on-screen button to start, allowing measurement of the time to first consumption. After the five minute ad libitum intake period, participants were instructed to stop eating and begin the ratings task, which required them to rate the sweetness, saltiness, familiarity, pleasantness and savouriness of all 6 items on a scale from 0 (Not At All Pleasant/Salty/Sweet/Familiar/Savoury) to 100 (Extremely Pleasant/Salty/Sweet/Familiar/Savoury). All remaining food and drink was reweighed to allow intake to be calculated. A web-camera (Logitech C170) was placed on top of the computer on which participants completed the intake tasks. The camera was angled down to capture the tray of foods placed in front of the participants. Participants were video-recorded throughout the intake session, allowing analysis of intake patterns including choice and latency of first food contact, number of selections, etc.

Participants were then given a debriefing questionnaire. This involved asking participants to state what they believed to be the purpose of the experiment and how this may have affected them in any way. Their height and weight was then measured followed by payment.

2.2.6.5. Statistics

Power calculations based on Birch et al. (1989)'s paper suggested a sample size of 52 participants.

The effects of a cue-manipulation on overall intake was assessed using independent measures ANOVA with two Conditions (CS+, CS-). Since it was expected that men would eat more than would women, gender was also included (Gender: Males, Females).

To test whether the effects of the cue manipulation on grams consumed depended on the nature of the food consumed, a 2 (Food type: total grams of sweet foods eaten, total grams of savoury foods eaten) by Condition was performed.

To test whether awareness of the conditioned stimulus and food association affected the amount consumed a 2 (awareness: aware, unaware) by Condition independent measures ANOVA was performed on total grams eaten.

To test whether the cue-manipulation modified liking for the snack foods at test, a 6 (Food type: water drink, squash drink, chocolate buttons, mini cheddars, pretzels, flapjacks) by Condition mixed measures ANOVA was performed on pleasantness ratings.

Table 2.2. Test items' nutritional information.

<i>Item</i>	<i>Amount (g's)</i>	<i>Total calories</i>	<i>Carbohydrates (of which sugars)</i>	<i>Sodium (equivalent as salt)</i>
Water	300ml	0	0	0
Robinsons Squash Orange and Mango	300ml (1:5 parts of concentrate to water as recommended by the manufacturer)	4.8	0.42	0.06
Cadbury's chocolate buttons	60	318	33.6	0.14
McVities Mini Cheddars	50	258	2.2	1.2
Sainsbury's Pretzels, Salted	30	118	0.5	0.45
Sainsbury's flapjacks	130	566	40.95	0.72

2.3. Results

2.3.1. Intake.

Participants in the CS+ condition ate significantly more ($413.95 \text{ grams} \pm 26.82$) than did those in the CS- condition (342.89 ± 26.87 ; $F(1, 40) = 4.24$, $p = .046$, $\eta^2 = .08$). Males (455.64 ± 28.27) ate significantly more than did females (301.21 ± 25.34 ; $F(1, 40) = 16.63$, $p < .001$, $\eta^2 = .29$), but there was no significant interaction between condition and gender ($F(1, 40) = 1.79$, $p = .19$). No participants correctly guessed the purpose of the experiment and so none were removed.

When analysed in terms of calories consumed the same results were obtained; main effect of condition ($F(1, 40) = 5.40$, $p = .025$, $\eta^2 = .12$), and gender ($F(1, 40) = 12.60$, $p \leq .001$, $\eta^2 = .23$), with no interaction between the two, ($F(1, 40) = 0.70$, $p = .826$).

A mixed measures ANOVA revealed a significant effect of food type ($F(1, 42) = 68.44$, $p < .001$, $\eta^2 = .61$). Across groups, participants consumed significantly more sweet (234.31 ± 14.78) than savoury foods (138.27 ± 9.52 ; $F(1, 42) = 68.44$, $p < .001$, $\eta^2 = .61$), and those in the CS+ group ate significantly more than did those in the CS- condition ($F(1, 42) = 4.07$, $p = .05$, $\eta^2 = .08$).

There was a trend for a significant interaction between group and type of food eaten ($F(1, 42) = 3.56$, $p = .066$, $\eta^2 = .06$): CS+ and CS- groups did not significantly differ on the amount of savoury food they consumed ($t(43) = 1.125$, $p = .27$), but those in the CS+ condition consumed significantly more sweet food than did those in CS- ($t(43) = 2.08$, $p = .04$, effect size $r = .30$: Figure 2.2].

When these data were analysed with intake converted to calories, there was a significant main effect of condition ($F(1, 42) = 4.10$, $p < .05$, $\eta^2 = .13$), and food type ($F(1, 42) = 18.16$, $p < .001$, $\eta^2 = .74$). However, the interaction between condition and food type eaten no longer approached significance ($F(1, 42) = 1.69$, $p > .05$).

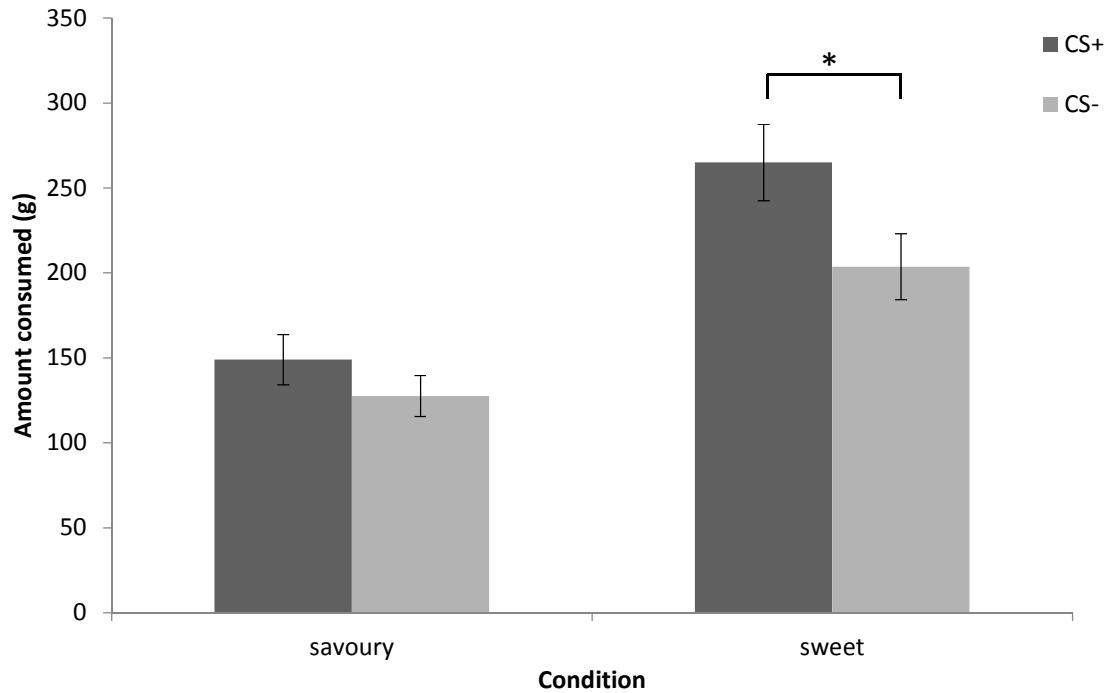


Figure 2.2. Amount consumed (g) of the sweet and savoury items in the two cue conditions. Data are mean ± 1 SEM, CS+ $n = 22$, CS- $n = 23$. * $p < .05$

2.3.2. Contingency.

Participants were defined as 'aware' if they explicitly knew what stimulus was associated with which taste in the conditioning phase. For example, the CS+ condition had to explicitly state that the CSsweet stimulus was paired with a sweet taste.

Although 'aware' participants ate marginally less ($M = 351.96 \pm 36.88$) than unaware participants (387.30 ± 28.59) the ANOVA revealed no significant difference between the two ($F(1, 41) = .57, p = .45$). There was no significant interaction between awareness and condition ($F(1, 41) = .14, p = .71$). The same results were obtained when analysed by calories consumed.

Whilst there was no significant effect of contingency awareness on overall consumption it may have affected intake of different foods (e.g. sweet over savoury). A 2 (Food type: total grams of sweet foods eaten, total grams of savoury foods eaten) by Awareness and Condition mixed measures ANOVA showed no significant main effects or interactions (p 's $> .05$).

2.3.3. Participant characteristics

A further confound would have been differences in appetitive state at time of testing. To assess this, the appetite rating made prior to intake were contrasted between conditions, and

theses did not differ significantly between conditions ($t(43) = 1.21, p = .234$). Similarly, tested conditions did not differ in age ($t(43) = .12, p = .905$) or BMI ($t(43) = .63, p = .535$).

2.3.4. Behavioural analysis

The latency to first food selected, first food selected and number of selections made were coded by the experimenter from the video-playback of each eating session, with 10% of the videos also being coded by a second experimenter to check reliability. Cohen's k revealed extremely strong agreement between experimenters ($k = 0.95, SE = 0.03$).

To test whether the cue influenced the latency to first food selected a 2-way (Condition) ANOVA was performed. It revealed no significant difference in the latency to select the first food between CS conditions [$F(1,25) = 0.55, p = .70$: CS+ 5.2 ± 0.7 , CS- 6.2 ± 0.7 sec].

To test whether the cue influenced which food participants selected first choices were divided into whether the first item was a sweet or savoury food. A chi-square test showed that there was no significant effect of condition on first food selected ($X^2(1) = 0.024, p = .923$). There was, also, no significant effect of condition on which food was most selected ($X^2(1) = 0.00, p = .999$).

Table 2.3. The effects of conditioned stimuli on behavioural measurements.

Behavioural Measurement	Test	P value	Significant?
Latency to first food selected	ANOVA	.70	NS
First food selected	Chi-square	.923	NS
Food most selected	Chi-square	.999	NS

Whilst the cue did not have a significant effect on which food was selected first, the cue may have had a sustained effect over the course of the five minutes. A 5 (Time: Number of selections in minute 1, Number of selections in minute 2, Number of selections in minute 3, Number of selections in minute 4, Number of selections in minute 5) x 2 (Food type: sweet, savoury) by Condition and Gender mixed-measures ANOVA was performed. See Figure 2.3. The ANOVA revealed a significant main effect of time ($F(1, 14) = 7.11, p = .018, \eta^2 = .23$). More selections were made in the first minute than in the third minute and fourth minute ($p < .05$) but no different from the second and fifth minute (p 's $> .05$). No other main or interaction effects reached significance (p 's $> .05$). There was also a significant main effect of food type ($F(1, 14) = 4.14, p = .005$). Savoury ($3.12 \pm .17$) was chosen more often than sweet ($2.39 \pm .12$).

There was a trend for an interaction between Time, condition and gender ($F(4, 56) = 2.52, p = .051, \eta^2 = .34$). Split by gender, there was no significant difference between CS+ and CS- on any time point for number of sweet or savoury selections (p 's $> .05$). Females in the CS+ condition (3.55 ± 1.81) made significantly more selections for sweet foods in the second minute than those in the CS- group ($1.77 \pm .93$) ($t(22)=3.10, p = .005$, effect size $r = .55$). The opposite was true for savoury selections in the second minute with CS- (3.08 ± 1.80) selecting it significantly more often than CS+ ($1.73 \pm .90$) ($t(22)= 2.25, p = .04$, effect size $r = .43$). For females in the CS+ condition ($2.64 \pm .92$) made significantly more selection for sweet foods in the fourth minute than those in the CS- condition (1.23 ± 1.48) ($t(22)=2.73, p = .01$, effect size $r = .50$).

All other effects and interactions were not significant (p 's $> .05$).

Whilst there was no significant difference between the number of selections, the increased intake may be due to the CS+ condition taking larger bite sizes. There was no significant difference between the conditions in regards to average bite size (total grams eaten/total number of selections) ($t(41)= 1.11, p = .28$; CS+ = 14.49 ± 4.54 , CS- = 12.85 ± 5.12).

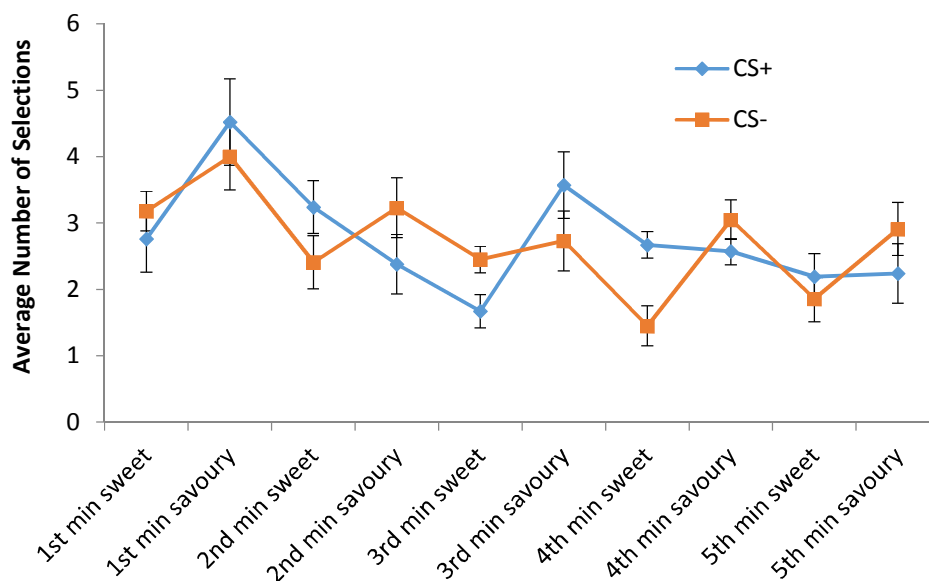


Figure 2.3. Number of sweet and savoury food selections by the two cue conditions across the five minute intake test. Data are mean ± 1 SEM, CS+ $n = 22$, CS- $n = 23$.

2.3.5. Hedonic and sensory evaluations of the test snacks.

There was no significant main effect of condition ($F(1, 48)= 2.29, p = .14$), and no significant interaction between condition and food type, ($F(5, 240)= .33, p = .90$). There was a significant

difference between the foods in terms of pleasantness ($F(5, 240) = 11.93, p < .001, \eta^2 = .20$). Post Hoc tests showed that chocolate (89 ± 2) was liked significantly more than water (62 ± 4), squash (74 ± 3), and pretzels (71 ± 3) (p 's $< .01$), but no differently from cheddars (79 ± 3) or flapjacks (85 ± 3) (p 's $> .05$). Water was liked equally to squash and pretzels (p 's $> .05$), but significantly less than cheddars and flapjacks (p 's $< .01$). Squash was liked equal to cheddars and pretzels (p 's $> .05$) but significantly less than flapjacks ($p = .046$). Cheddars were liked equally to pretzels and flapjacks (p 's $> .05$). Pretzels were liked significantly less than flapjacks ($p = .008$).

Given that odours which have been paired with sweet tastes subsequently smell sweeter (Stevenson, Prescott & Boakes, 1995), the hypothesis that the presence of the sweet cue would enhance the perceived sweetness of the snacks in the CS+-cued condition was also tested using a 4 (Food type: water drink, squash drink, chocolate buttons, mini cheddars, pretzels, flapjacks) \times 2 (Condition: CS+, CS-) mixed measures ANOVA. However, there was no significant main effect of condition ($F(1, 48) = .17, p = .68$), nor a significant interaction between condition and food item ($F(3.63, 174.39) = .62, p = .64$) for these sweetness ratings.

There were significant differences in perceived sweetness between food items ($F(3.63, 174.39) = 184.56, p < .001, \eta^2 = .79$). Post hoc tests showed the sweet food items (squash (75 ± 3), chocolate (88 ± 2), and flapjacks (80 ± 2)) were rated as sweeter than the savoury food items (water (11 ± 3), cheddars (19 ± 4), pretzels (18 ± 3)) (p 's $< .05$).

2.4. Discussion

The aim of this study was to investigate the effect of a sweet-associated cue on subsequent *ad libitum* intake, latency to first food selected and first and most food chosen compared to a cue not associated with a neutral taste. The key finding was that a cue paired with a sweet taste increased consumption compared to a cue paired with a neutral taste. This extends the previous literature showing that a food-availability cue increased intake (Birch et al., 1989), by showing that a cue associated with a hedonic sweet taste (but without ingestion) increased intake compared to a cue associated with a neutral taste. A novel and interesting finding in the present study is that the increase in intake tended to be specific to the taste the cue had been associated with; the sweet-paired cue significantly increased intake of sweet items. This supports previous research in animals that have shown cue-specificity in cue-potentiated feeding (Galarce, Crombag & Holland, 2007).

The finding that a cue associated with the isolated hedonic component of reward (a liked sweet taste without any ingestion) increased consumption compared to a cue associated with a neutral solution suggests that for Pavlovian conditioning to occur in this instance all that was needed was a brief co-experience of a cue and a hedonic sweet taste, even in the absence of actual ingestion. However, this study utilised only participants who found the taste of sweet hedonically rewarding; using those who find the taste of sweet aversive would further dissociate this hedonic component. A minority of people show decreased liking to increasing sweetness, or their liking peaks at low concentrations (Ekman & Akesson, 1965; Pangborn, 1970). Although this hedonic response applies to a variety of sources of sweet tastes, such as sucrose, glucose and fructose, sweet-liker status does not affect responses to other tastes such as salt (Looy, Callaghan, & Weingarten, 1992), and is consistent not only to sweet solutions but also to solid sweet foods (Moskowitz, Kluter, Westerling, & Jacobs, 1974). Dissociation of this hedonic reaction to sweet tastes by utilising the naturally occurring sweet-likers and sweet-dislikers will be used to investigate the theory that cues associated with hedonic rewards increase non-specific eating.

Whilst the findings show cue-controlled feeding, this could be due to three things: CS+ potentiation, CS- inhibition, or both. To dissociate these three possibilities experiment two should include an additional control condition, possibly with a no cue present condition (to gauge a measure of general intake in the *ad libitum* situation).

Interestingly, contingency awareness did not appear to play a role in affecting cue-potentiated feeding contradicting previous research (Birch et al., 1989). Whilst conclusions from a null result are limited this result may not be too surprising following Brunstrom's (2004) suggestion that contingency awareness may not be needed in specialist systems such as the gustatory system. However, there are a number of issues with using categorical methodology to measure contingency awareness. Firstly, by chance alone one fifth of participants rated as aware will have been due to chance. Secondly, it lacks detail that can be provided by continuous measures, such as visual analogue scales (VAS). By using VAS we can obtain knowledge unavailable with categorical data, such as relative confidence in awareness decisions.

The present study sought to expand the findings from experiment one by exploring cue-potentiated feeding in adults through associations between novel visual cues and a taste that was either liked or disliked. Participants (who were either sweet-likers or sweet-dislikers) learned to associate a sweet taste with a novel, visual image using a triangle-disguised training method (Stevenson, Prescott, & Boakes, 1995; Yeomans et al., 2006). Post training participants were given free-access to sweet and savoury snack foods labelled with either a cue paired with

a sweet taste, a cue paired with a neutral taste or no cue present. Wanting and liking ratings were, also, taken along with measures of contingency awareness.

It was predicted that when the cue that had been experienced with a sweet taste (CS_{sweet}) was present in the free feeding situation sweet-liking participants would eat more overall, and in particular more of the sweet flavoured items, compared to sweet-liking participants who had the cue associated with a neutral taste present, or no cue present. Also, when the sweet-paired cue was present sweet-liking participants would eat more of all of the foods compared to the sweet-disliking participants with the sweet-paired cue present.

Experiment 2

2.5. Method

For method synopsis see table 2.1.

2.5.1. Design

In addition to the sweet CS₊, and the CS₋ condition in Experiment one, we included a control condition (No Cue) who also experienced the same procedure but no visual cue was present in the taste task.

2.5.2. Participants

One hundred and twenty participants consented to participate in a study on “taste perception”. Initially they completed the taste screening session (see below) however 48 participants were not eligible to complete the main session due to showing neutral or erratic ratings. Seventy-two healthy females participated in the main study. One participant was excluded for not following experimenter instructions. The remaining 71 females were aged between 18 and 57 ($M = 21.15$, $STD = 5.66$) and BMI of 22.10 ± 2.98 . All were currently staff or students at the University of Sussex. Females, only, were chosen due to the lack of an effect of gender in Exp.1. The experimental protocol was approved by The University of Sussex’s Ethics Committee and conformed with British Psychological Society rules on ethical conduct. Participants gave written informed consent at the start of the test session, and they were compensated by payment of £7 and/or awarded course credits on completion of the study. Because a history of dieting may decrease sensitivity in flavour-based learning (Brunstrom, Downes, & Higgs, 2001) and alter responses to food cues (relative to unrestrained eaters) (Fedoroff, Polivy & Herman, 1997), participants first completed the Three Factor Eating

Questionnaire (Stunkard & Messick, 1985): participants scoring seven or higher on the Restraint scale were not invited to take part in the study. Participants with a history of eating disorders, who were diabetic, had specific allergies to any ingredients used in the study, who smoked more than five cigarettes per week or who presently used prescription medication (excluding the contraceptive pill) were excluded.

2.5.3. Taste Screening Session

To distinguish sweet-likers from sweet-dislikers, potential participants attended a short test session at least one day before the main test and rated two samples of 10% sucrose solution and two samples of water on pleasantness, sourness, sweetness, bitterness and saltiness using visual analogue scales, end-anchored with 'Very pleasant' (scored 100) and 'Very unpleasant' (scored 0) for rating flavour pleasantness and 'Extremely' (scored 100) and 'Not at all' (scored 0) for the other ratings. The label for the dimension evaluated was written above the centre of each line. Ratings were made using Sussex Ingestive Pattern Monitoring (SIPM2.013, University of Sussex). 10ml samples of each solution were refrigerated and brought to room temperature prior to tasting. Consistent with previous research (Yeomans et al., 2006), participants were deemed sweet-likers if their average ratings on two sucrose solutions were at least 60pt on the pleasantness and sweetness scale and dislikers if they also rated sweetness above 60pt but rated pleasantness below 45pt.

2.5.4. Stimuli

2.5.4.1. Conditioned stimuli. The stimuli were the same as in Experiment one.

2.5.4.2. Unconditioned stimuli. The stimuli were the same as in Experiment one.

2.5.5. Control of hunger state

The same lunch as used in Experiment one was given.

2.5.6. Procedure

2.5.6.1. Standard lunch. The same procedure was used as in Experiment one.

2.5.6.2. Conditioning phase. The same procedure was used as in Experiment one.

2.5.6.3. Contingency Awareness. Based on previous research (Wardle, Mitchell & Lovibond, 2007), participants completed a contingency awareness questionnaire in which participants were presented a CS and asked "Was this picture paired with a SALTY/SOUR/SWEET/NO TASTE AT ALL taste most of the time in this experiment?" (order counterbalanced across participants). They rated their answer on a 100mm VAS scale, end-anchored with 'Definitely Was' (scored

100) and 'Definitely wasn't' (scored 0). Participants were considered aware of the CS-US contingency if they rated the paired US as more likely to have been paired with the paired CS than any of the unpaired USs. For example, to be aware of the sweet-paired CS a participant would have to give a higher rating for that CS on the sweet scale than the sour, salty or no taste at all scales. They were then instructed to wait in the waiting room for 30 minutes whilst the intake test phase of the experiment was set up in the same room.

2.5.6.4. Testing. At the start of the intake test, participants were informed that they would complete a taste evaluation task. Participants were informed using SIPM that prior to conducting a series of VAS ratings of foods and drinks over a five minute period, that during this period they must try each food and drink at least once, but that they could eat and drink as much as they wanted as any remaining food would have to be discarded. The experimenter then provided participants with a tray containing four bowls (two with sweet foods and two with savoury foods [see Table 2.2]), one glass of sweet drink and one glass of neutral drink) on a tray. For participants in the CS+ condition ($n = 22$), the outside of the bowls and glasses contained the CSsweet stimulus sticker, while in the CS- condition ($n = 22$) the bowls and glasses contained the CSneutral cue on the outside ($n = 20$).

Participants were instructed that they will be rating these foods and drinks on their visual properties, however they must not consume the food or drinks yet. Without trying the samples, participants then answered 'how much do you want some of Sample X now?' and 'how pleasant would it be to experience a mouthful of Sample X now?' for each of the samples in a random order. They rated their answer on a 100mm VAS scale, end-anchored with 'Extremely' or '(scored 100) and 'Not At All' (scored 0). After completing hunger and thirst ratings, participants then completed a series of questions after sampling one mouthful of each food and drink on the same VAS scale. The questions included 'how salty/sweet/familiar/savoury/pleasant is Sample X?' and 'how much do you want more of Sample X?'. Then, after more hunger and thirst ratings, participants were informed that they would now have a few minutes to try as much of the food and drinks as they want and if they needed more to call the experimenter.

Participants clicked an on-screen button to start, allowing measurement of the time to first consumption. After five minutes SIPM informed them to stop eating and complete hunger and thirst ratings. After they had finished the remaining food was weighed.

A web-camera (Logitech C170) was placed on top of the computer on which participants completed the intake tasks. The camera was angled down to capture the tray of foods placed in front of the participants. Participants were video-recorded throughout the intake session,

allowing analysis of intake patterns including choice and latency of first food contact, number of selections, etc.

Participants were then given a debriefing questionnaire. This involved asking participants to state what they believed to be the purpose of the experiment and how this may have affected them in any way. Their height and weight was then measured followed by payment.

2.5.6.5. Statistics

The effects of the cue-manipulation on overall intake (see Figure 2.4) was assessed using independent measures ANOVA with three Conditions (CS+, CS-, No Cue). Since we predicted Liker Status (Likers, Dislikers) would also affect consumption, it was also included.

To test whether intake was affected by awareness of the association between the cues and the tastes they were previously associated with, a 2 (Contingency Awareness: Aware, Unaware) by Condition and Liker Status independent measures ANOVA was conducted on total grams eaten.

The effects of the cue manipulation of consumption of the different types of food (sweet or savoury) was assessed a 2 (Food type: Total grams eaten of sweet foods, total grams eaten of savoury foods) by Condition and Liker Status mixed measures ANOVA

To test whether intake of the different foods was affected by awareness of the cue-taste associations, a 2 (Food type: Total grams eaten of sweet foods, Total grams eaten of savoury foods) by Condition, Contingency Awareness, and Liker Status mixed measures ANOVA was conducted.

To test whether Liker Status and the cue-manipulation modified hunger for the snack foods at test, a Condition by Liker Status independent measures ANOVA was conducted on absolute hunger scores after first seeing, but not consuming, the test items. A Condition by Liker Status independent measures ANOVA was conducted on absolute hunger scores after consuming the test items.

A 2 (Wanting ratings: first wanting ratings prior to intake, second wanting ratings after consumption of one mouthful) by Condition, Contingency Awareness, and Liker Status mixed measures ANOVA was conducted on absolute scores. A 2 (Liking Ratings: first liking ratings prior to intake, second liking ratings after consumption of one mouthful) by Condition, Contingency Awareness, and Liker Status mixed measures ANOVA was conducted on absolute scores.

A Condition by Liker Status independent measures ANOVA was performed on average bite size (total grams eaten/total selections).

2.6. Results

2.6.1. Intake.

There was no significant main effect of condition ($F(2, 65) = .45, p = .642$). However, the data contained two significant outliers ($z = 2.92$) due to eating excessive amounts. All data from this participant were excluded from further analyses. After removal of this outlier, there was no significant effect of condition ($F(2, 64) = 1.03, p = .361$). There was no significant difference in amount eaten depending on Liker Status with Likers eating marginally more (221 ± 19) than Dislikers (212 ± 19) ($F(1, 58) = .24, p = .63$). There was no significant interaction between condition and Liker Status ($F(2, 64) = .37, p = .695$).

When analysed by calories consumed the same results were obtained; no significant main effect of condition ($F(2, 64) = .80, p = .454$), and Liker Status ($F(1, 64) = .13, p = .725$), with no significant interaction between the two ($F(2, 64) = 1.15, p = .323$).

One possible confound would have been if participants had guessed the study purpose and so conformed to the expected outcome. However, when participants who had correctly guessed the true purpose of the experiment ($n=2$) were removed from the analysis similar results were obtained; main effect of condition ($F(2, 62) = 1.03, p = .365$), and Liker Status ($F(1, 62) = .07, p = .786$), with no interaction between the two ($F(2, 62) = .40, p = .673$).

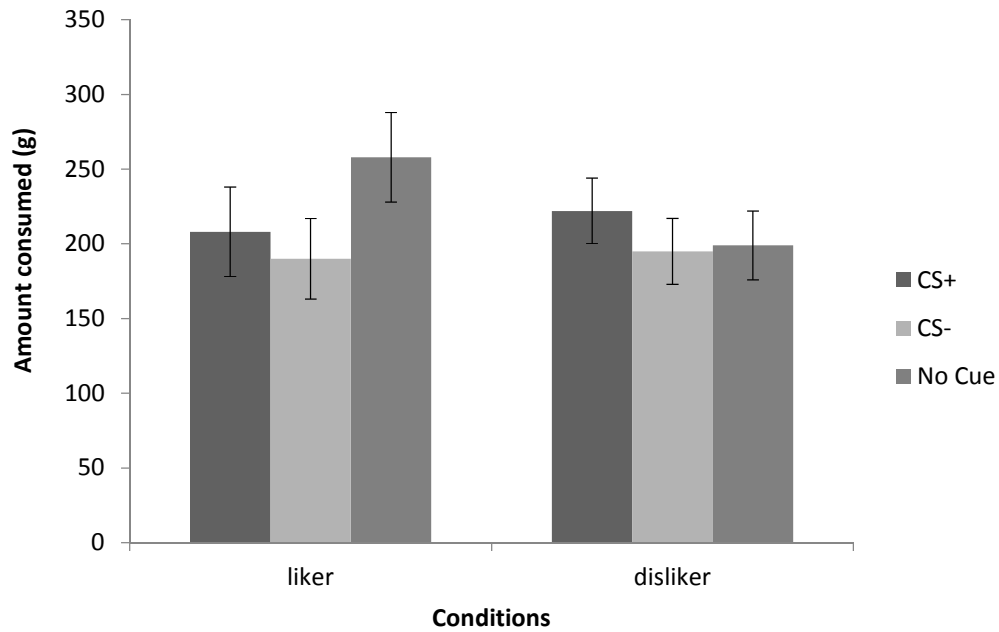


Figure 2.4. Amount consumed (g) of the sweet and savoury items in the three cue conditions, separated by Liker Status. Data are mean ± 1 SEM, CS+ Liker $n = 10$, CS+ Disliker $n = 12$, CS- Liker $n = 11$, CS- Disliker $n = 11$, No Cue Liker $n = 9$, No Cue Disliker $n = 11$.

2.6.2. Effects of contingency awareness.

There was no significant overall effects of condition ($F(1, 33) = 1.37, p = .251$) or Liker Status ($F(1, 33) = .32, p = .577$), and no significant interaction between Liker Status and condition ($F(1, 33) = .19, p = .668$) (see Figure 2.5). There was also no significant effect of contingency awareness ($F(1, 33) = 1.14, p = .293$), and none of the interactions involving awareness were significant. When analysed by calories consumed the same results were obtained (p 's $> .05$).

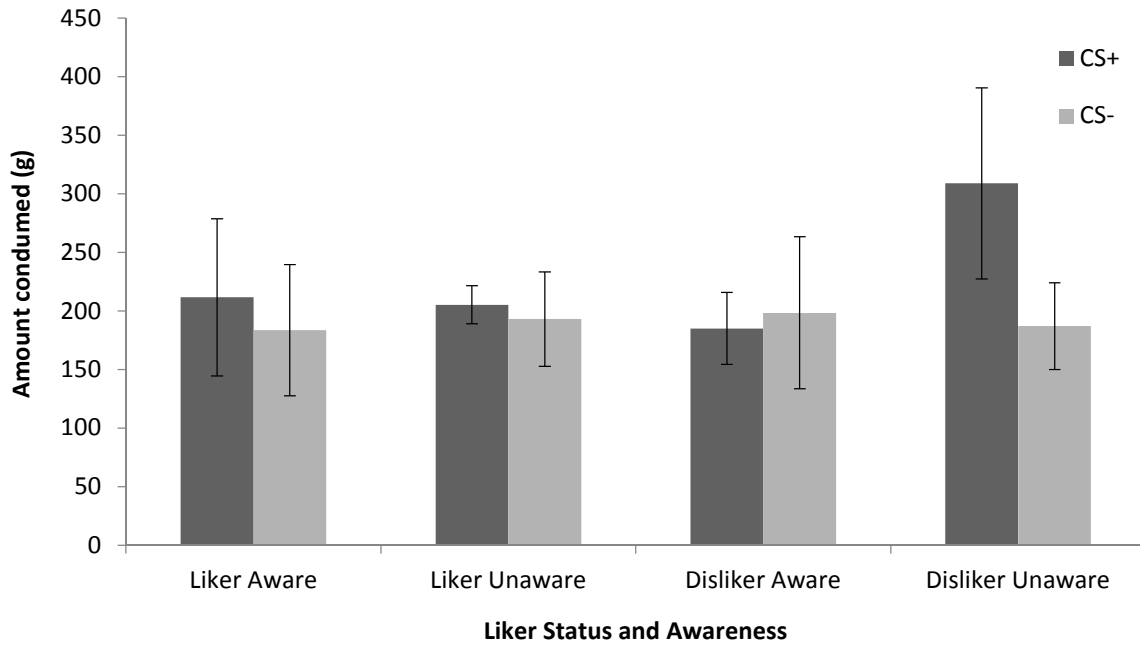


Figure 2.5. Amount consumed (g) in the two cue conditions, separated by Liker Status and Contingency Awareness. Data are mean ± 1 SEM, CS+ Liker Aware $n = 5$, CS- Liker Aware $n = 3$, CS+ Liker Unaware $n = 5$, CS- Liker Unaware $n = 6$, CS+ Disliker Aware $n = 8$, CS- Disliker Aware $n = 4$, CS+ Disliker Unaware $n = 3$, CS- Disliker Unaware $n = 5$.

Note. Contingency Awareness (CA) can only be compared with CS- and CS+ groups.

2.6.3. Intake of different foods.

There was a significant main effect of food type ($F(1, 64) = 11.35, p = .001, \eta^2 = .14$) (see Figure 2.6). Post hoc tests showed that more grams of sweet foods (131 ± 11) were eaten than savoury foods (85 ± 8) ($p = .001$). There was no significant effect of condition ($F(2, 64) = 1.03, p = .361$) or Liker Status ($F(1, 64) = .12, p = .736$), and no significant interaction between Condition and Liker Status ($F(2, 64) = .37, p = .695$). There was no interaction between type of food eaten and Condition ($F(2, 64) = 1.02, p = .368$). All other interactions were not significant (p 's $> .05$).

When analysed by calories the results remained not significant (p 's $> .05$).

There was a significant effect of food type ($F(1, 33) = 7.15, p = .012, \eta^2 = .14$). There was no significant main effect of Contingency Awareness ($F(1, 33) = 1.14, p = .293$). There was no significant interaction between Contingency Awareness and Condition ($F(1, 33) = 1.21, p = .280$). All other main effects and interactions were not significant (p 's $> .05$).

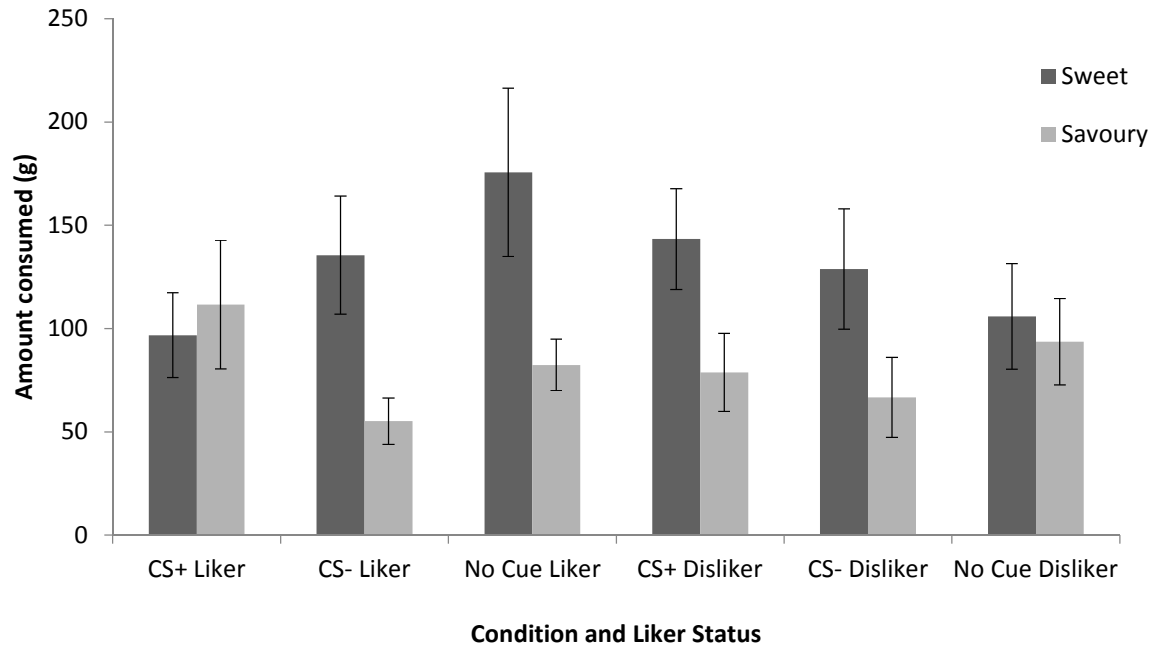


Figure 2.6. Amount consumed (g) of the sweet and savoury items in the three cue conditions, separated by Liker Status. Data are mean ± 1 SEM, CS+ Liker $n = 10$, CS- Liker $n = 11$, No Cue Liker $n = 9$, CS+ Disliker $n = 12$, CS- Disliker $n = 11$, No Cue Disliker $n = 11$. * $p < .05$

2.6.4. Hedonic and sensory evaluations of the test snacks.

2.6.4.1. Hunger Ratings prior to conditioning.

A potential confound would have been difference in appetitive state at time of testing. To assess this, the appetite ratings made prior to condition phase were contrasted between conditions, this showed that the CS+ condition rated themselves as significantly more hungry (41 ± 5) than the CS- (30 ± 5) and No Cue (21 ± 6) conditions ($F(2, 64) = 3.64, p = .032, \eta^2 = .11$). However, there was no significant difference between conditions at the start of the intake session ($F(2, 67) = .30, p = .745$). BMI did not differ significantly between conditions ($F(2, 67) = .47, p = .628$).

2.6.4.2. Hunger Ratings on first seeing, and after eating, the test samples.

Condition ($F(2, 64) = .19, p = .827$), and Liker Status ($F(1, 64) = .48, p = .493$), had no significant effect on hunger ratings after being presented with the foods and drinks. There was no significant interaction between Liker Status and condition ($F(2, 64) = 1.83, p = .168$). See Table 2.4.

After undertaking the five min intake period, participants completed another set of hunger ratings. This was analysed to investigate whether cues, rather than increasing initial motivation to eat, cause a sustained motivation to eat by not reducing hunger. Condition ($F(2, 64) = .59, p = .560$), and Liker Status ($F(1, 64) = 2.28, p = .136$), had no significant effect on hunger ratings after the 5min intake period. There was no significant interaction between Liker Status and condition ($F(2, 64) = .12, p = .884$).

Table 2.4. Hunger ratings (VAS) on first seeing, and after eating, the test samples in the two cue conditions separated by Liker Status. Brackets show ± 1 SEM.

	CS+ Liker	CS+ Disliker	CS- Liker	CS- Disliker	No Cue Liker	No Cue Disliker
<i>Hunger ratings on first seeing the test items</i>	64.50 (9.87)	78.67 (3.91)	64.82 (6.11)	72.91 (5.03)	71.89 (7.25)	65.36 (6.40)
<i>Hunger ratings after 5min intake period</i>	22.70 (7.97)	30 (6.24)	24.18 (8.52)	28.73 (6.21)	27.22 (8.23)	35.91 (7.07)

2.6.4.3. Wanting for Sweet Foods.

To test whether Liker Status and the cue manipulation affected explicit wanting of the snack foods, prior to intake participants were asked ‘how much do you want some of Sample X now?’. After tasting one mouthful of each item participants were then asked ‘how much do you want more of Sample X?’. Wanting ratings of sweet foods prior to consumption (67 ± 3) did not significantly differ from wanting ratings of sweet foods after tasting one mouthful of each food (64 ± 3) ($F(1, 33) = 2.96, p = .10$).

Rated wanting for sweet foods did not differ significantly between sweet likers and dislikers ($F(1, 31) = .15, p = .70$), nor between the CS+ and CS- test conditions ($F(1, 33) = 1.71, p = .20$). There was trend for a main effect of awareness ($F(1, 33) = 3.37, p = .08$), with aware participants rating the foods as significantly less sweet (61.71 ± 3.71) than unaware participants (71.33 ± 3.70). However, none of the interactions between Liker Status, condition and awareness were significant (all p 's > 0.05).

2.6.4.4. Liking for Sweet Foods.

To test whether Liker Status and the cue-manipulation modified liking for the snack foods at test, prior to intake participants were asked 'how pleasant would it be to experience a mouthful of Sample X now?'. After tasting one mouthful of each item participants were then asked 'how pleasant is Sample X?'.

Across conditions there was a trend for liking ratings to increase from first rating prior to intake (73.35 ± 2.25) to second rating after one mouthful of the sweet foods (77 ± 2) ($F(1, 33) = 3.99$, $p = .06$, $\eta^2 = .11$). Across foods, there was a trend for the CS+ condition to rate liking higher (79 ± 3) than the CS- condition (72 ± 3) ($F(1, 33) = 3.17$, $p = .09$, $\eta^2 = .09$). This did not significantly change from first to second ($F(1, 31) = .88$, $p = .36$).

Unaware participants average liking ratings were significantly higher (80 ± 3) than aware participants (71 ± 3) ($F(1, 33) = 5.28$, $p = .03$, $\eta^2 = .15$). Liking ratings did not change according to awareness ($F(1, 33) = 2.19$, $p = .15$). All other Liker Status, awareness, liking and condition interactions were not significant (p 's $> .05$).

2.6.4.5. Sweetness Ratings.

It was investigated whether the presence of the sweet cue would enhance perceived sweetness of the snacks in the CS+-cued condition. Sweetness ratings ('how sweet is Sample X?') were made after tasting one mouthful of each test item. A 2 (Sweetness rating: sweetness rating of sweet foods, sweetness ratings of savoury foods) by Condition, Contingency Awareness, and Liker Status mixed measures ANOVA was conducted on absolute scores

Across all participants, sweet foods were rated as significantly sweeter (81 ± 2) than the savoury foods (17 ± 2) ($F(1, 33) = 685.01$, $p < .001$, $\eta^2 = .96$). Aware participants rated the foods generally as significantly sweeter (53 ± 2) than Unaware participants (45 ± 2) ($F(1, 31) = 7.44$, $p = .01$, $\eta^2 = .19$). There was no main effects of condition nor Liker Status on sweetness ratings (p 's $> .05$).

There was a significant sweet rating and condition interaction ($F(1, 33) = 4.87$, $p = .04$, $\eta^2 = .14$). Participants in the CS+ condition rated the sweet foods as sweeter (84 ± 2) than those in the CS- condition (79 ± 2). They rated the savoury foods as less sweet (14 ± 2) than those in the CS- condition (20 ± 3). T-tests suggested there was no difference between conditions in sweet ratings of sweet foods ($t(42) = 1.15$, $p = .26$), nor for sweet ratings of savoury foods ($t(38.48) = .39$, $p = .70$).

There was a significant main sweet rating and Liker Status interaction ($F(1, 33) = 11.57$, $p = .002$, $\eta^2 = .27$). Likers rated the sweet foods as less sweet (78 ± 2) than Dislikers (85 ± 2)

($t(62) = 1.18, p = .24$). Likers rated the savoury foods as sweeter (22 ± 3) than Dislikers (12 ± 3) ($t(62) = .57, p = .57$).

There was a significant sweet rating by Contingency Awareness interaction ($F(1, 33) = 24.43, p < .001, \eta^2 = .44$). Aware participants rated the sweet foods as less sweet (79 ± 2) than unaware participants (84 ± 2) ($t(37) = .65, p = .52$). Aware participants rated the savoury foods as sweeter (27 ± 3) than unaware (7 ± 3) ($t(37) = 3.86, p < .001$, effect size $r = .54$).

There was a trend for a sweet rating, Liker Status and CA interaction ($F(1, 33) = 3.77, p = .06, \eta^2 = .11$). Aware Likers rated sweet foods as less sweet (75 ± 4) than unaware Likers (81 ± 3) ($t(17) = .69, p = .50$). Aware Likers rated savoury foods as sweeter (36 ± 5) than unaware Likers (8 ± 4) ($t(17) = 5.27, p < .001$, effect size $r = .79$). Aware Dislikers rated sweet foods as less sweet (84 ± 3) than unaware Dislikers (86 ± 4) ($t(18) = .92, p = .37$). Aware Dislikers rated savoury foods as sweeter (19 ± 3) than unaware Dislikers (6 ± 5) ($t(18) = 1.70, p = .11$).

All other interactions were not significant (p 's $> .05$).

2.6.5.1. Behavioural Analysis.

The latency to first food selected, which food was first selected and number of selections made were coded by the experimenter from the video-playback of each eating session, with 10% of the videos also being coded by a second experimenter to check reliability. Cohen's k revealed extremely strong agreement between experimenters ($k = 0.92, SE = 0.09$).

To test whether the cue influenced the latency to first food selected a Condition, Contingency Awareness, and Liker Status mixed measures ANOVA was performed. There was no significant main effect of condition of latency to first food selected ($F(2, 30) = .01, p = .92$). No significant effect of Liker Status ($F(1, 30) = .02, p = .90$), nor of awareness ($F(1, 30) = .57, p = .46$). All other interactions were not significant (p 's $> .05$).

To test whether the cue influenced which food participants selected first choices were divided into whether the first item was a sweet or savoury food. A chi-square test showed that Condition had no significant effect on which food item participants selected first ($X^2(2) = .73, p = .70$). Liker Status ($X^2(1) = 1.40, p = .24$), and contingency awareness ($X^2(1) = .03, p = .87$), also had no significant effect on which item was chosen first. There was, also, no significant effect of condition on which food was most selected ($X^2(2) = .43, p = .81$).

Latency to first item selected was not significantly correlated with any explicit wanting ratings for savoury or sweet foods (p 's $> .05$).

First food item selected was not predicted by which food was rated as most pleasant before, or which was rated as being wanted the most (p 's $> .05$). However, after tasting one

mouthful of the foods the first item selected was significantly related to which food (sweet or savoury) participants rated as the most pleasant ($X^2(1) = 3.56, p = .06$), and which food (sweet or savoury) was rated as being wanted the most ($X^2(1) = 9.75, p = .002$).

2.6.5.2. Sweet and Savoury Selections by time periods.

Whilst the cue did not have an effect on which food was selected first, the cue may have had a sustained effect over the course of the five minutes. A 5 (Time: Number of food selections in minute 1, Number of food selections in minute 2, Number of food selections in minute 3, Number of food selections in minute 4, Number of food selections in minute 5) x 2 (Food: sweet, savoury) by Condition mixed-measures ANOVA was performed. The ANOVA revealed a significant main effect of time ($F(3.43, 209.32) = 27.50, p < .001, \eta^2 = .11$). There was significantly more selections in the first minute ($2.69 \pm .14$) than the second ($2.04 \pm .15$), third ($1.66 \pm .15$), fourth ($1.60 \pm .14$) and fifth ($1.44 \pm .12$) (p 's $< .001$). There was also a trend for a significant difference between the two foods in terms of number of times they were selected ($F(1, 61) = 3.40, p = .07, \eta^2 = .09$), with savoury foods ($2.12 \pm .19$) being selected more often than sweet foods ($1.65 \pm .14$). Whereas sweet selections did not decrease over time ($F(3.58, 225.59) = 1.63, p = .17$), the number of savoury selections did ($F(3.46, 218.10) = 18.68, p < .001, \eta^2 = .30$). The number of savoury selections in the first minute ($3.52 \pm .35$) was significantly greater than in any other minute (second, $2.14 \pm .26$; third, $1.88 \pm .26$; fourth $1.75 \pm .23$; fifth, $1.28 \pm .17$) (p 's $< .001$). Savoury selections in the second minute was also significantly more often than in the fifth minute ($p = .03$). All other comparisons were not significant (p 's $> .05$). Number of selections of sweet and savoury foods only differed in the first minute ($t(63) = 3.25, p = .002$). See Table 2.5.

All other effects and interactions were not significant (p 's $> .05$).

Table 2.5. Number of sweet and savoury food selections by the two cue conditions across the five minute intake test.

Group	Sweet 1 st min	Savoury 1 st min	Sweet 2 nd min	Savoury 2 nd min	Sweet 3 rd min	Savoury 3 rd min	Sweet 4 th min	Savoury 4 th min	Sweet 5 th min	Savoury 5 th min
CS+	2.50 (.44)	3.09 (.61)	2.32 (.39)	2.27 (.54)	1.27 (.33)	2.00 (.50)	1.45 (.40)	1.59 (.34)	1.86 (.45)	1.32 (.32)
CS-	1.36 (.30)	3.18 (.49)	1.55 (.31)	1.95 (.45)	1.41 (.34)	1.50 (.44)	1.41 (.28)	1.45 (.36)	1.05 (.36)	1.14 (.28)
No Cue	1.65 (.42)	4.35 (.69)	1.95 (.38)	2.20 (.344)	1.60 (.44)	2.15 (.43)	1.45 (.38)	2.25 (.50)	1.85 (.33)	1.40 (.26)

2.6.6. Average Bite Size.

If the cue manipulation did not have an effect in regards to the number of selections participants made it may have influenced how much participants consumed per mouthful. There was no significant main effect of condition ($F(2, 58) = 1.03, p = .36$), or liker status ($F(1, 58) = .004, p = .95$), or interaction ($F(2, 58) = .78, p = .46$).

2.6.7. Exploratory analysis

Due to generally low intake, across conditions, compared to Experiment 1 an exploratory analysis was performed. Using the lower bound of the 95% CI of the CS- condition in Experiment 1 (382 calories eaten) as a minimum amount eaten for inclusion, a 3-way (Condition) independent measures ANOVA was performed on total calories eaten (9 people in the CS+, 10 in the CS- and 10 in No Cue). The results showed that there was a significant difference in the amount eaten by the different conditions ($F(2, 26) = 3.741, p = .037, \eta^2 = .22$). Specifically, the CS+ condition (611 ± 36) ate significantly more than the CS- condition (484 ± 35) ($p = .05$) but not from the No Cue condition (499 ± 35) ($p = .11$). The CS- and No Cue did not differ ($p = 1$).

In grams, there was no significant difference between conditions ($F(2, 26) = 1.77, p = .19$).

2.7. General Discussion

The aim of these experiments was to investigate the effect of food-associated cues on subsequent *ad libitum* intake, latency to first food selected and first and most food chosen compared to cues not associated with food.

Experiment one showed that a cue associated with a sweet taste increased consumption compared to a cue with a neutral taste. However, experiment two contradicted this finding by finding no evidence of cue potentiated feeding, which also contradicted previous research (Grant & Milgram, 1973; Galarce, Crombag & Holland, 2007; Holland & Gallagher, 2003; Galarce & Holland, 2009; Valle, 1968; Detke, Brandon, Weingarten, Rodin, & Wagner, 1989; Birch, McPhee, Sullivan, & Johnson, 1989). One potential explanation of the lack of cue potentiated feeding is due to a change in methodology between Experiment one and Experiment two. In Experiment two, prior to intake, participants rated wanting and liking questions sequentially, which were not asked in Experiment one. They then ate one mouthful before answering the same wanting and liking questions. By asking participants to try one mouthful of each food and drink prior to the five minute intake period, we may have contradicted the cognitive expectations of the US elicited by the conditioned stimulus (Reiss, 1980). Upon seeing the conditioned stimulus associated with a sweet-taste participants' elicited expectations may have been altered by actual sensory experience. For example, upon seeing the conditioned stimulus associated with a sweet-taste dislikers may have thought the food to be less pleasant than if they had tasted it, however upon tasting the food it disconfirmed expectations and potentially produced a compensatory effect (shown by the increased consumption of dislikers in the CS+ condition compared to likers in the same condition). However, this explanation seems unlikely due to the lack of a difference between likers and dislikers in pleasantness ratings before a mouthful of the foods were ingested. Another explanation could be due to the generally low intake across participants, despite the inclusion of only low restraint participants. In comparison to Experiment one, females in the CS+ condition ate, on average, 130 less calories and in the CS- condition 30 less calories. As shown in the exploratory analysis, it would appear that once a certain amount of food is eaten the conditioned stimulus has an additive effect.

Data from both experiments suggests that participants do not need to have explicit knowledge of the learnt relationship between stimuli and outcome for this to affect behaviour shown by the lack of effect of contingency awareness on food consumed. Although this contrasts with previous research (Birch et al., 1989) this does support the findings from

Experiment one and other research (Brunstrom, 2004) indicating a lack of necessity for awareness in specialist systems such as the gustatory system.

The conditioned stimuli did have an effect on explicit liking ratings. In experiment two the CS+ condition rated the sweet food as liked more than the CS- condition, regardless of Liker Status. The different findings for wanting and liking suggests that, despite suggestions from previous research (Zandstra, Weegels, Van Spronsen, & Klerk, 2004; Finlayson, King, & Blundell, 2007b), these explicit measures may investigate different components despite the liking and wanting ratings being highly correlated for sweet and savoury food and drinks (all p 's < .001).

There was no evidence from either experiment for the latency to which participants selected their first food item to be influenced by conditioned stimuli, contradicting previous research (Birch et al., 1989). Similarly the conditioned stimuli did not influence which type of food, sweet or savoury, was selected first. Whilst the stimuli did not affect implicit wanting measures such as these, they also did not influence explicit wanting ratings shown in experiment two. The lack of significant correlation between the latency scores and explicit wanting ratings may mean they are measuring different aspects of wanting or that explicit wanting scores are not a reliable measure of actual wanting (Finlayson et al., 2007b), though the latter argument is debateable in this study as explicit wanting ratings were highly predictive of which food participants would select first.

Although which food was selected first was not influenced by the conditioned stimuli, it was influenced by which food was subjectively rated as being most pleasant prior to tasting the food items. It was also highly associated with which food was rated as being wanted the most. This suggests that whilst the stimuli may have an effect on food selection other factors are seem ably more important in this task.

However, it remains unknown what is driving this cue-potentiated feeding. It is hypothesized that after associating the stimuli with the hedonic reward incentive is attributed to the stimulus through Pavlovian conditioning so that it comes to be 'wanted' and elicits wanting behaviour (Robinson & Berridge, 1993). Berridge and Robinson (1998) themselves suggest that 'wanting' is measured by voluntary intake or preference tests. Whilst the data from experiment one support the former, the lack of a cued effect on first food choice selected limits our ability to suggest the increased intake was due to 'wanting' elicited by the stimuli. However, the first choice selected may not show the predicted effect due to our methodology. Specifically, if participants know they are able to make multiple selections over the course of five minutes then 'wanting' may have been overridden by other psychological processes. A

different effect may have been observed if the participants were only allowed to make one selection rather than multiple over the course of the *ad libitum* eating period.

There was no effect of Liker Status on amount eaten of sweet or savoury foods. Although sweet dislikers do not like the taste of a 10% sucrose solution and research has shown that this dislike translates to solid foods, people have a higher sweetness level for their breakpoint of foods compared to sucrose solutions (Moskowitz, Kluter, Westerling, & Jacobs, 1974). The lack of effect may be due to the sweet foods being simply not sweet enough to reach dislikers' breakpoint. This could, also, explain the lack of significant difference between likers and dislikers in pleasantness ratings of the foods and drinks.

In conclusion, the lines of research in these experiments may provide interesting and novel methodology to disentangle the hedonic component of cue potentiated eating, however findings from these experiments are contradictory and future work is needed. The experimental methodology used here may be beneficial in investigating the liking component of reward however more research is needed to investigate whether this is truly dissociated from wanting.

Chapter 3: The Effect of Discriminative Stimuli on Intake in a Free-Feeding Situation.

3.1. Introduction

Homeostatic systems are widely believed to play a less prominent role in the development and maintenance of obesity than external, non-homeostatic stimuli (Mela, 2006; Schachter, 1971; Hill & Melanson, 1999). Appetitive behaviour, even under the conditions of satiety, can be prompted by the presence of excitatory, external food cues (Weingarten, 1983; Galarce, Crombag & Holland, 2007; Birch, McPhee, Sullivan, & Johnson, 1989) or the removal of inhibitory cues (Todd, Winterbauer & Bouton, 2012). However these external stimuli do not affect appetitive behaviour in purely a Pavlovian manner; most eating situations involves some form of instrumental response. To fully understand the determinants of eating behaviour an observation of instrumental performance is critical (Miller, Bailey, & Stevenson, 1950).

Whilst Pavlovian stimuli come to signal *what* and *when* outcomes will occur, stimuli that signal *whether* an outcome will occur are called Discriminative Stimuli (DS). These stimuli signal which response one must produce in order to obtain a specific outcome (Michael, 1982). These discriminative stimuli are distinct from Pavlovian stimuli indicating reinforcement availability; rather it signals specifically which type of behaviour is relevant to obtain reinforcement. Whilst the organism may cognitively form DS-outcome associations, in reality the stimulus rarely alone predicts the outcome; only the combination of the stimulus and a specific instrumental response yields reinforcement. Similar to Pavlovian stimuli (Petrovich, Ross, Gallagher, & Holland 2007), discriminative stimuli are specific to the outcome it has been associated with (Bonardi, 1989). Additionally, DS' ability to control responding will extend to new instrumental responses that have earned the same reinforcer but has little impact on responses associated with a different reinforcer (Colwill & Rescorla, 1988).

Substantial research in non-human animals has shown that the DS elicits behaviour which is consistent with the animal having an expectation of the specific outcome, which in turns becomes a stimulus to predict which response is needed to produce that outcome. For example, Brodigan and Peterson (1976) trained pigeons that one response following one DS was consistently rewarded with food whilst a different response was required following another DS to be rewarded with water. Compared to an inconsistent group in which food and

water were presented on an irregular, intermixed, equiprobable basis following either stimulus, the pigeons trained with consistent DS developed reinforcer-specific expectancies in the presence of the DS (through performance of reinforcer-specific behaviour). Similarly, using a fixed consecutive number schedule (where a certain number of responses are required before one response on another will be reinforced), cues signalling when the switch of responses was required increased efficiency in subjects compared to a cue signalling nothing (Kruse, Overmier, Konz, & Rokke, 1983).

In humans, Pavlovian contingencies between stimuli and reinforcers are most likely embedded within discriminative instrumental contingencies (Hogarth, Dickinson & Duka, 2003). Predominately DS research has focused on drug-related behaviours. For example, in a study by Hogarth et al. (2003) a DS signalled that a key press would allow the participant two puffs of a cigarette whereas another DS signalled the same key press would allow the person to blow into a carbon monoxide monitor. Participants showed discriminative learning, by increased visual attention towards the smoking paired stimulus compared to the non-smoking paired stimulus, but only males expressed this (potentially explaining some of the gender disparity in smoking-related behaviours [See Hitchman & Fong, 2011]).

In regards to ingestive behaviour in humans, the research has been more limited. To investigate satiety, Havermans, Janssen, Giesen, Roefs, and Jansen (2009) allowed participants to consume 250ml of chocolate milk prior to pressing a button for points they could trade for a certain reward (either earning points for crisps (one group) or chocolate (another group)). They observed a food-specific decrease in both wanting (measured by number of presses on a progressive ratio schedule) and liking (measured by explicit subjective visual analogue scale (VAS) ratings) of chocolate compared to crisps. Similarly, participants presented with the same food choice showed a more rapid decrease in responding than those presented with a variety of food (Ernst & Epstein, 2002). Using a progressive ratio measure to investigate incentive salience (Berridge, & Robinson, 2003), Epstein, Truesdale, Wojcik, Paluch and Raynor (2003) found a difference between deprived and fed participants in their motivation to eat ('wanting'), however no difference between hedonic ratings of snack food.

However, to our knowledge, no food research in humans has investigated the effect of a DS on intake: previous research has instead focussed on outcome or incentive evaluation (Havermans et al., 2009). If a DS signals when a response-outcome is applicable and that outcome is food, then in the presence of food that DS should lead to an increase in food-related behaviour compared to behaviour without the presence of the stimulus or in the presence of a stimulus predicting the absence of reward (*DS-*). In the present study

participants had the opportunity to associate one stimulus with one R to receive a sweet reward (chocolate) whereas another stimulus with a different response would lead to a savoury reward (crisp). Other stimuli predicted nothing (*DS-*). Participants were then placed in a free-feeding situation with either the sweet-associated stimulus, the savoury-associated stimulus, the *DS-* or *No Cue* stimulus present, and intake of a variety of savoury and sweet foods was measured. As well as contingency awareness, explicit ‘wanting’ and ‘liking’ were measured.

It was predicted that when the cue that had been experienced with chocolate (*DSchoc*), if the correct response was produced, was present in the free feeding situation participants would eat more overall, and in particular more of the sweet flavoured items, compared to those participants presented with the same snacks labelled with the cue associated with not earning a food reward (*DS-*), or when *No Cue* was present (*No Cue*). Additionally, when the cue that had been experienced with crisps (*DScrisp*), if the correct response was procured, was present participants would eat more overall, in particular more of the savoury flavoured items, compared to when the cue associated with no rewards or *No Cue* was present.

Experiment 1

3.2. Method

Table 3.1. Method synopsis.

Method	Task
<i>Control of hunger state</i>	Participants refrained from eating for 2 hours.
<i>Conditioning phase</i>	Participants associated stimuli with foods on screen.
<i>Contingency awareness test</i>	Presentation of stimuli and asked which foods were associated with each stimulus.
<i>Intake test</i>	Participants were presented with a selection of food items with the conditioned stimuli on the bowls containing the food/drink.

3.2.1. Design

A between-participants design contrasted the effects of visual cues on measures of food-related intake following prior association of *de novo* cues with the chance to earn food rewards. Four conditions were tested: two where these cues had been associated with winning either a sweet (chocolate, *DSchoc*) or savoury (Chips/Crisps, *DScrisp*) reward, one where cues were associated with the absence of food (*DS-*) and a *No-Cue* control.

3.2.2. Participants

Sixty-four healthy men and women consented to take part in a study described as ‘cognitive performance on sensory evaluations’. However, data from one participant were excluded due to computer error during the snack intake test. The 63 remaining participants (31 male) had a mean age of 21.6 years (range 18-51) and BMI of 23.32 ± 0.49 kg/m².

The experimental protocol was approved by The University of Sussex’s Ethics Committee and conformed with British Psychological Society rules on ethical conduct. Participants gave written informed consent at the start of the test session, and they were compensated by payment of £5 and/or awarded course credits on completion of the study.

Because a history of dieting may decrease sensitivity in flavour-based learning (Brunstrom, Downes, & Higgs, 2001) and alter responses to food cues (relative to unrestrained eaters: Fedoroff, Polivy & Herman, 1997), participants first completed the Three Factor Eating Questionnaire (Stunkard & Messick, 1985): participants scoring seven or higher on the Restraint scale were excluded. Likewise, potential participants with a history of eating disorders, who were diabetic, had specific allergies to any ingredients used in the study, who smoked more than five cigarettes per week or who presently used prescription medication (excluding the contraceptive pill) were excluded.

3.2.3. Stimuli

3.2.3.1. Test stimuli. The visual cues were adapted from a series of images constructed by Kuwayama (1973) and previously used in cue-based food learning (Laane, Aru, & Dickinson, 2010). To select images that were distinct and neutral, a pilot study was conducted where 12 participants rated the novelty and hedonic appeal of 29 images. Four images rated as high in novelty and neutral in liking were selected as stimuli for use in this study (see Figure 3.1). The specific pairing of stimuli and outcome was counterbalanced across test conditions.

3.2.3.2. Reward stimuli. There were two food stimuli presented, both of which were pictures of common snacks (crisps or chocolate: see Figure 3.1). These images were 8cm x 8cm.

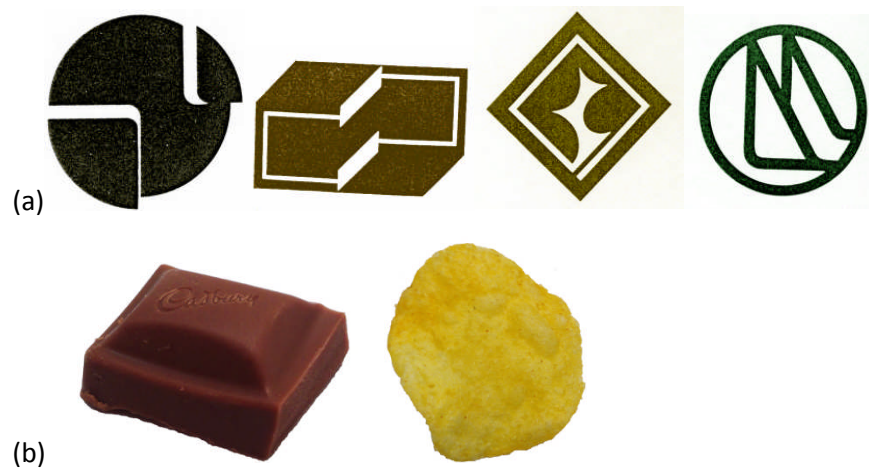


Figure 3.1. The four target discriminative stimuli (a) and two food images (b) used during the operant training phase.

3.2.4. Control of hunger state

Hunger can alter brain activation (LaBar et al., 2001), in areas associated with stimulus salience attribution (Amaral, Price, Pitkänen, & Carmichael, 1992). For this reason, participants were asked to refrain from eating and to drink only water for 2 hours prior to starting the experiment. Similar to previous research (Yeomans, Spetch, & Rogers, 1998) to ensure abstinence participants were informed a saliva swab may be taken at the start of the session to confirm compliance. This swab was never actually taken, but acted as an incentive to comply with these restrictions.

3.2.5.1. Procedure

Participants arrived at the laboratory between 1400-1700 for the start of the test day. Participants indicated their hunger and thirst on separate 100pt line scales from 0 (not at all hunger/ thirst) to 100 (extremely hungry/thirsty) disguised as a mood rating questionnaire, presented using Susses Ingestive Pattern Monitoring (SIPM, version 2.013, University of Sussex). These questions were integrated into a larger mood questionnaire measuring 'How energetic/calm/clearheaded/strong is your desire to eat/much do you think you could eat/full/tired/headachy do you feel right now?' on separate 100pt scales from 0 (not all all) to 100 (extremely). All testing was conducted in small air-conditioned cubicles at the University of Sussex.

3.2.5.2. Operant Conditioning phase. Participants were instructed that they would be playing a computer game (programmed using E-Prime version 1.2). The initial instructions read:

“In this game you may collect points by pressing the ‘*m*’ or ‘*z*’ key. By pressing these keys you may earn points for crisps or chocolate. You will have 30 seconds per trial. You may earn multiple points per trial. Pay attention! Not every ‘*z*’ or ‘*m*’ key press will earn you a point. You may have to press more than once. Press SPACE to begin.”

After participants had verbally instructed that they understood the instructions, they were left in the room to complete the task. During this task one of four target images were presented in the centre of the screen for 30 seconds. Above this was written “‘press ‘*z*’ or ‘*m*’””. During this time participants had to press either the ‘*z*’ or ‘*m*’ key to earn a reward. They were not informed which button was the correct choice. When participants correctly paired the target symbol with the appropriate button press (Table 3.2) and pressed a certain number of times (operating on a variable-ratio schedule of 5 presses), they were presented with a specific food picture (Table 3.2). These images were presented in the bottom left corner of the screen for two seconds before being removed. During this two second period no key presses were counted. After 30 seconds the DS was removed from the screen simply showing a blank screen for 10 seconds. The four DS’ were presented in a random order before repeating in a different random order four more times (20 presentations in total). Two images (*DS-*) were merely presented on-screen for 30seconds and the pressing of either key produced no reward (called extinction stimuli). The food images presented and key altered between images. One DS signalled a chocolate stimulus if the ‘*z*’ key was pressed a certain number of times. One DS signalled a crisp stimulus if the ‘*m*’ key was pressed a certain number of times. All images, reward and keys were counterbalanced across participants (for example, for one participant Image one would signal chocolate if the ‘*z*’ key was pressed a certain number of times whereas for another participant Image two would have signalled chocolate with if the ‘*m*’ key was pressed).

3.2.5.3. Contingency Awareness. Using similar methodology to previous research (Wardle, Mitchell & Lovibond, 2007), participants were presented with each image and asked “Was this picture paired with winning NOTHING/CHOCOLATE/CRISPS most of the time in this experiment?”. They rated their answer on a 100pt VAS, end-anchored with ‘Definitely Was’ (scored 100) and ‘Definitely wasn’t’ (scored 0), programmed using E-Prime. Participants were classified as aware of the *DS*-reward contingency if they rated the paired reward as more likely to be paired with the paired DS than any of the unpaired rewards. For example, to be aware of the chocolate-paired DS a participant would have to give a higher rating for that DS on CHOCOLATE scale than the NOTHING or CRISPS scales. They were then instructed to wait in the

waiting room for 30minutes whilst the intake test phase of the experiment was set up in the same room.

3.2.5.4. Snack intake test. At the start of the intake test, participants were instructed that they would now be completing a taste evaluation task. After completing hunger and thirst ratings disguised as a mood questionnaire using SIPM, participants were informed that they would be conducting a series of ratings on a selection of foods over a five minute period, that during this period they must try each food at least once, but that they could eat as much as they wanted as any remaining food would have to be discarded. The experimenter then provided participants with a tray containing four bowls (two with sweet foods and two with savoury foods [see Table 3.2]). After verbally instructing that they had understood the instructions the experimenter brought the test items into the room.

Table 3.2. Test items' nutritional information.

<i>Item</i>	<i>Amount (g's)</i>	<i>Total calories</i>	<i>Carbohydrates (of which sugars)</i>	<i>Sodium (equivalent as salt)</i>
Cadbury's chocolate	120	636	68	0.28
Walkers Ready Salted Crisps	30	158	0.12	0.56
Sainsbury's Pretzels, Salted	40	157	0.72	0.60
Sainsbury's flapjacks	120	522	37.8	0.7

Participants were randomly assigned to one of four conditions; Chocolate condition (*DSchoc*) ($n = 16$), Crisp condition (*DScrisp*) ($n = 16$), *DS-* condition (*DS-*) ($n = 15$), and *No Cue* condition (*No Cue*) ($n = 16$). For the *DSchoc* condition, the outside of the bowls contained stickers (4cm x 4cm) of the image associated with winning chocolate. Similarly, the *DScrisp* condition had the image associated with winning crisps on all items, while the *DS-* condition had one of the two non-winning cues present (There was no difference between which of the two *DS-* was present on intake and so results were collapsed for this condition). The *No Cue* condition had *No Cue* on the test items.

There were two differing versions of the taste evaluation task. In Intake Method One participants were informed that they will be rating these foods and drinks on their visual properties, and not to consume the food. Participants then answered 'how much do you want

some of Sample X now?’ and ‘how pleasant would it be to experience a mouthful of Sample X now?’ for each of the samples in a random order. They rated their answer on a 100pt VAS scale, end-anchored with ‘Extremely’ or ‘(scored 100)’ and ‘Not At All’ (scored 0). Participants then completed hunger and thirst ratings. Participants completed a series of ratings (‘how salty/sweet/familiar/savoury/ pleasant is Sample X?’ and ‘how much do you want more of Sample X?’) after sampling one mouthful of each food on the same VAS scale. After completing these ratings participants were informed that they would have a few minutes to familiarise themselves with the samples and if more was needed to call the experimenter. Once ready participants clicked the screen (this was to control onset in order to measure reaction times). After five minutes they were instructed (using SIPM) to stop eating and to complete another set of hunger and thirst ratings. After completion the remaining food was weighed.

In Intake Method Two participants conducted the same procedure however they completed the five minute eating period prior to conducting the wanting/liking ratings after sampling one mouthful of each food. This was to investigate whether tasting a mouthful of each food, as was the procedure in our previous Pavlovian experiment (Chapter two), prior to intake contradicted the cognitive expectations elicited by the CS (Reiss, 1980). To investigate this half of the participants in the current study conducted Intake Method 1 and half conducted Intake Method Two.

A web-camera (Logitech C170) was placed on top of the computer on which participants completed the intake tasks. The camera was angled down to capture the tray of foods placed in front of the participants. Participants were video-recorded throughout the intake session, allowing analysis of intake patterns including choice and latency of first food contact, number of selections, etc.

Participants were finally given a debriefing questionnaire. This involved asking participants to state what they believed to be the purpose of the experiment and how this may have affected them in any way. Their height and weight was then measured followed by payment.

3.2.6. Statistics

Power calculations were based on the previous experiments mentioned in this thesis, both those conducted in chapter 2 and those referenced in Chapter 1. The power calculation indicated the total sample size needed 64 participants.

Initially, the effects of cue-manipulation on overall intake were assessed using independent measures ANOVA with four Conditions (*DSchoc*, *DScrisp*, *DS-*, *No Cue*). Since it

was expected that men would eat more than would women, gender was also included (Gender: Males, Females). It was predicted that participants in the conditions associated with food (*DSchoc*, *DScrisp*) would consume more than those in the *DS-* and *No Cue* conditions. To test whether any overall effects of cue on intake were specific to the relevant cue-associated foods, a 4 (Food type eaten: chocolate, crisps, pretzels, flapjacks) by Condition and Gender mixed measures ANOVA was performed, with the prediction that the associated cue would specifically increase intake of the relevant food (greater chocolate intake in *DScrisp* condition, etc.). To test whether explicit awareness of the stimulus-reward association affected these consumption measures, a 2 (Contingency Awareness: Aware, Unaware) by Condition independent measures ANOVA was conducted on total grams consumed. It was predicted that aware participants in the *DSchoc* and *DScrisp* conditions would eat more than the unaware participants in the same conditions.

The effects of the cue-manipulation on explicit liking and wanting both prior to intake and after tasting one mouthful were assessed by a 4 (food type) by condition and gender mixed-measures ANOVA. It was predicted that the *DSchoc* and *DScrisp* conditions would cause increased wanting ratings of the test food items.

To test whether the cue influenced the latency to first food selected a 4-way (Condition) ANOVA was performed on time to first food chosen. It was predicted that the *DSchoc* and *DScrisp* would elicit shorter latencies to first food selections.

3.3.1. Results

The critical aspect of the conditioning phase of the procedure was the development of associations between discriminative stimuli and their respective rewards. Fourteen participants did not win any rewards during the conditioning task. Due to this they were not included in any of the following analyses. These ‘non-winners’ were not included in the *DS-* condition as not winning during two out of four stimuli is a very different experience from not winning to all stimuli (and thus not knowing that any stimuli were rewarding). Forty-nine participants remained; 12 in the *DSchoc* condition, 11 in the *DScrisp* condition, 15 in the *DS-* condition, and 11 in the *No Cue* condition. Data was log-transformed where significant Levene’s indicated it appropriate. To remove problems with sphericity, significance levels were adjusted using Greenhouse-Geisser or Huynh Feldt where appropriate.

3.3.2. Intake Analysis

There was no significant main effect of condition ($F(3, 49) = 1.15, p = .341, \eta^2 = .08$). However, the data contained two significant outliers ($z = 2.56$ and 2.63). All data from these participants were excluded from further analyses. After removal of these outliers, there was a significant effect of condition ($F(3, 39) = 3.57, p = .023, \eta^2 = .14$). Participants in the *DSchoc* condition ($139.67 \text{ grams} \pm 10.84$) ate significantly more than those in the *DS-* condition (90.04 ± 10.71) ($p = .014$), but not differently from the *DScrisp* (117.76 ± 11.37) and the *No Cue* conditions (112.83 ± 11.77) (p 's $> .05$). Men (146.15 ± 7.43) ate significantly more than women (90.25 ± 7.31) ($F(1, 39) = 24.30, p < .001$) but there was no interaction between condition and gender ($F(3, 39) = 2.20, p = .104$).

When analysed in terms of calories consumed similar results were obtained; significant main effect of condition ($F(3, 39) = 3.54, p = .023, \eta^2 = .21$), and gender ($F(1, 39) = 23.40, p < .001, \eta^2 = .38$), with no significant interaction between the two ($F(3, 39) = 2.12, p = .113$).

One possible confound would have been if participants had guessed the study purpose and so conformed to the expected outcome. However, when participants who had correctly guessed the true purpose of the experiment ($n=2$) were removed from the analysis similar results were obtained; significant main effect of condition ($F(3, 37) = 3.84, p = .017, \eta^2 = .24$), and significant gender ($F(1, 37) = 21.05, p < .001, \eta^2 = .36$), with no significant interaction between the two ($F(3, 37) = 2.49, p = .075$).

Intake Version had no significant effect on any intake measure (p 's $> .05$) and so was not included in any of the following intake analyses.

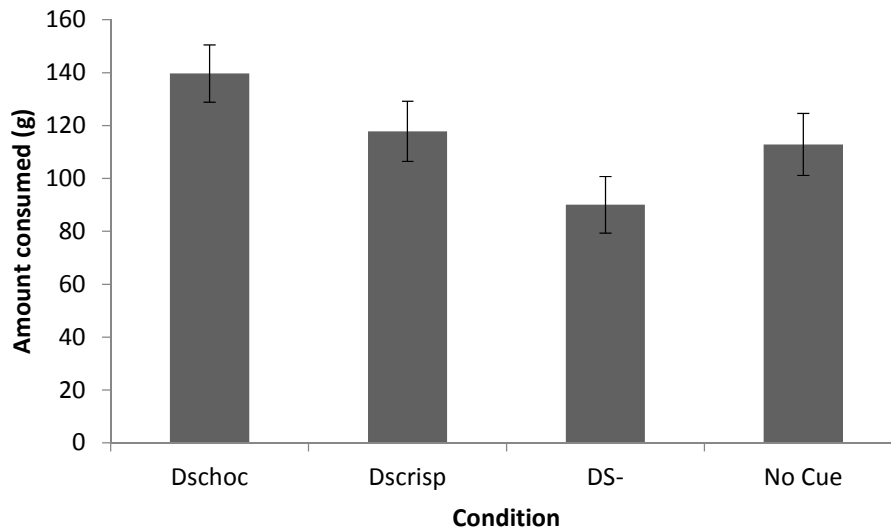


Figure 3.2. Amount consumed (g) in the four cue conditions. Data are mean \pm 1 SEM, DSchoc $n = 12$, DScrisp $n = 11$, DS- $n = 15$, No Cue $n = 11$.

A mixed measures ANOVA revealed there was a significant main effect of food type eaten ($F(3, 117) = 32.18, p < .001, \eta^2 = .46$). Chocolate ($36.89 \text{ grams} \pm 3.54$) was eaten significantly more than crisps (19.69 ± 1.20) and pretzels (13.07 ± 1.24) (p 's $< .05$), but not significantly differently from flapjacks (45.38 ± 3.52) ($p = .511$). All other comparisons were significantly different ($p < .01$). See Figure 3.3.

There was however no significant interaction between condition and food type eaten ($F(9, 117) = .67, p = .737$), between food type eaten and gender ($F(3, 117) = .07, p = .98$) or a three-way interaction ($F(9, 117) = .32, p = .97$). When collapsed in this way, the overall effect of condition was no longer significant ($F(3, 39) = 1.96, p = .14$), probably because of the large impact of food type on intake. There was a significant effect of gender ($F(1, 39) = 18.20, p \leq .001, \eta^2 = .39$), with males (35.65 ± 1.93) eating significantly more than females (21.86 ± 2.02), but no significant condition by gender interaction ($F(3, 39) = 1.53, p = .222$).

When analysed in terms of calories consumed the same results were obtained; significant main effect of food type ($F(3, 117) = 40.32, p < .001, \eta^2 = .46$), and condition ($F(3, 39) = 2.02, p = .127$), and gender ($F(1, 39) = 18.24, p < .001, \eta^2 = .38$). All other interactions were not significant (p 's $> .05$).

A further potential confound would have been differences in appetitive state at the time of testing. To assess this, the appetite ratings made prior to conditioning phase were contrasted between groups, and these did not differ significantly between conditions ($F(3, 39) = .15, p = .93$). They also did not significantly differ prior to the intake test ($F(3, 39) = .60, p = .62$). Likewise, although groups overall did not differ in age or BMI as noted in the methods,

since 16 participants were excluded for the final analyses, demographic data were re-analysed in case spurious group differences were now apparent. The tested groups did not differ in age, but there was now a significant difference in BMI between conditions (see Table 3.3) ($F(3, 43) = 4.35, p = .009, \eta^2 = .23$). Participants in the *DSchoc* condition had a significantly lower BMI than those in the *DScrisp* condition and the *DS-* condition (p 's $< .01$). However, BMI was not significantly correlated with total grams consumed across conditions ($r = -.11, n = 61, p = .393$) or separated by condition (all p 's $> .05$).

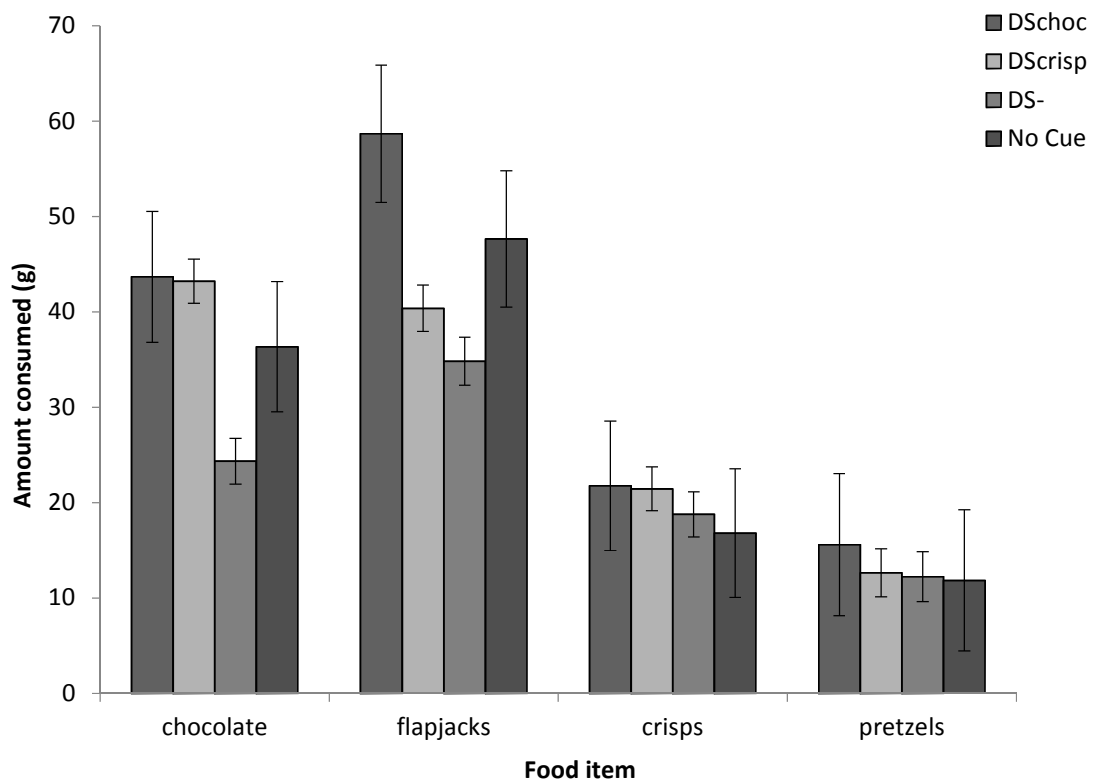


Figure 3.3. Amount consumed (g) of the different food items in the four cue conditions. Data are mean \pm 1 SEM, *DSchoc* $n = 12$, *DScrisp* $n = 11$, *DS-* $n = 15$, *No Cue* $n = 11$.

Table 3.3. BMI across the four cue conditions.

Condition	Mean (SE)
<i>DSchoc</i>	21.69* (.82)
<i>DScrisp</i>	25.77 (.85)
<i>DS-</i>	24.24 (.79)
<i>No Cue</i>	24.68 (.85)

* $p < .01$

3.3.3. Contingency Awareness

Participants were classified as aware of the *DS*-reward contingency if they knew which reward had been paired with the stimulus in their condition. For example, participants in the *DSchoc* condition had to explicitly rate the *DSchoc* stimulus as being paired with chocolate.

Including awareness reduced the overall significance of the effect of condition ($F(2, 30) = 3.16, p = .057, \eta^2 = .17$), but there was no significant effect of contingency awareness ($F(1, 30) = .47, p = .498$) nor a significant interaction between condition and contingency awareness status ($F(2, 30) = 1.12, p = .340$). Similar results were obtained when analysed in terms of calories consumed or by separate foods consumed.

3.3.4. Conditioning

To test whether the number of stimulus-reward associations in each condition during the conditioning phase affected intake during the snack test, a correlation was performed between the number of rewards won in each *DS+* condition and total grams consumed. Neither number of chocolate rewards won or crisp rewards won correlated significantly with overall intake (p 's $> .05$), or the amount eaten of the individual food items (all p 's $> .05$). Number of chocolate rewards won significantly correlated with number of crisp rewards won ($r(58) = .85, p < .001$).

Since all participants completed the same training and were then randomly allocated to intake conditions, there should have been no group differences in performance on the conditioning task between conditions. To test this, the number of rewards won were analysed using a 2 (Reward type won: Total chocolate rewards won, Total crisp rewards won) by Condition mixed measures ANOVA. The conditions did not significantly differ in the number of rewards won ($F(3, 29) = .37, p = .77$). There was no significant interaction between condition and type of rewards won ($F(3, 29) = .86, p = .47$). All other interactions were not significant (p 's $> .05$).

3.3.5.1. Hedonic and sensory evaluations of the test snacks

As well as modifying intake, the prior associations between visual cue *DS* and experience of reward during training might have affected the way participants experienced the snacks during the intake test. To test this, the ratings made during the snack test were analysed.

3.3.5.2. Expected Liking Prior to Intake

Prior to intake participants were asked 'how pleasant would it be to experience a mouthful of Sample X now?'.

Across conditions, there was a significant difference in the amount participants liked the different foods ($F(3, 117) = 13.97, p < .001, \eta^2 = .25$). Chocolate (85 ± 2) was liked more than crisps (76 ± 2), pretzels (66 ± 3), and flapjacks (69 ± 3). Chocolate was significantly more liked than the others (p 's $< .05$) but the others did not differ from each other. However, there was no significant main effect of condition ($F(3, 39) = 2.06, p = .121$), nor a significant main effect of gender ($F(1, 39) = .15, p = .70$).

There was however a significant interaction between liking of the different foods and gender ($F(3, 117) = 3.46, p = .019, \eta^2 = .10$). Chocolate was liked significantly more by females (89 ± 3) than males (80 ± 3) ($t(47) = 2.00, p = .05$, effect size $r = .28$). There was a trend for males to like pretzels (71 ± 4) more than females (60 ± 4) ($t(47) = 1.86, p = .07$, effect size $r = .26$). Males did not differ on liking for crisps (73 ± 3 and 78 ± 3 respectively) or flapjacks (68 ± 4 and 70 ± 5) (p 's $> .05$).

All other comparisons and interactions were not significant (p 's $> .05$).

3.3.5.3. Expected Wanting Prior to Intake

Prior to intake participants were asked 'how much do you want some of Sample X now?'.

Across conditions there was a significant difference in the amount participants wanted certain foods ($F(3, 117) = 9.55, p < .001, \eta^2 = .20$). Chocolate (81 ± 2) was rated as being wanted significantly more than pretzels (67 ± 3) and flapjacks (67 ± 3), p 's $< .001$, but not crisps (74 ± 3), $p = .14$. There was no significant main effect of gender ($F(1, 39) = .14, p = .711$), nor a significant main effect of condition ($F(3, 39) = 1.62, p = .199$).

There was a significant interaction between gender and wanting ratings ($F(3, 117) = 2.87, p = .039, \eta^2 = .07$). Males' and Females' ratings of chocolate (Males 78 ± 3 , Females 83 ± 3), crisps (74 ± 3 , 74 ± 4), and flapjacks (67 ± 4 , 68 ± 5) did not significantly differ (p 's $> .05$). However, pretzels were wanted significantly more by males (73 ± 4) than females (61 ± 4) ($t(47) = 1.97, p = .05$, effect size $r = .26$).

All other interactions were not significant (p 's $> .05$).

3.3.5.4. Explicit Liking

After tasting one mouthful of each item participants were then asked 'how pleasant is Sample X?'.

Across conditions, there was a significant difference in liking ratings of the different food items ($F(3, 117) = 7.27, p < .001, \eta^2 = .16$). Chocolate (87 ± 2) was liked significantly more than pretzels (68 ± 4) and flapjacks (75 ± 3) (p 's $< .05$), but not crisps (78 ± 3) ($p = .43$). Crisps were liked significantly more than pretzels ($p = .05$). All other ratings were not significantly different. There was no significant main effect of condition ($F(3, 39) = 1.72, p = .179$), nor a significant main effect of gender ($F(1, 39) = .08, p = .779$). All other interactions were not significant (p 's $> .05$).

3.3.5.5. Explicit Wanting

After tasting one mouthful of each item participants were then asked 'how much do you want more of Sample X?'.

Across conditions, there was a significant difference in wanting scores of the different food items ($F(3, 117) = 3.41, p = .020, \eta^2 = .08$). Chocolate (71 ± 3) and crisps (72 ± 4) were wanted more than pretzels (62 ± 4) and flapjacks (60 ± 4) but not significantly so (p 's $> .05$). There was no significant effect of condition ($F(3, 39) = 2.09, p = .118$), nor interaction with wanting ratings ($F(9, 117) = .55, p = .835$). There was no significant main effect of gender ($F(1, 39) = .17, p = .686$). All other interactions were not significant (p 's $> .05$).

3.3.5.6. Sweetness

After tasting one mouthful of each item participants were then asked 'how sweet is Sample X?'. Given that odours which have been paired with sweet tastes subsequently smell sweeter (Stevenson, Prescott & Boakes, 1995), the hypothesis that the presence of the chocolate associated cue would enhance the perceived sweetness of the snacks in the *DSchoc* cued condition was also tested using a 4 (Food type: sweetness rating for chocolate, sweetness rating for crisps, sweetness rating for pretzels, sweetness rating for flapjacks) by Condition and Gender mixed measures ANOVA was conducted on sweetness ratings.

Across conditions, the foods differed in rated sweetness ($F(3, 117) = 246.84, p < .001, \eta^2 = .86$). Post hoc test showed the sweet food items (chocolate (92 ± 1) and flapjacks (74 ± 3)) were rated as sweeter than the savoury food items (pretzels (17 ± 3) and crisps (12 ± 3)) (p 's $< .001$). There was no significant main effect of condition ($F(3, 39) = 1.45, p = .244$), nor a significant interaction between condition and sweetness rating ($F(9, 117) = 1.37, p = .21$). There was no significant main effect of gender ($F(1, 39) = .27, p = .605$). All other interactions were not significant (p 's $> .05$).

3.3.6. Behavioural analysis

Previous research (Birch et al., 1989) has indicated that conditioned stimuli elicit rapid conditioned responses to be produced. To investigate whether this happened to the discriminative stimuli the latency to first food selected, first food selected and number of selections made were coded by the experimenter from the video-playback of each eating session, with 10% of the videos also being coded by a second experimenter to check reliability. Cohen's k revealed strong agreement between experimenters ($k = 0.82$, $SE = 0.15$).

There was no significant effect of condition of latency to first selection ($F(3, 35) = 1.65$, $p = .20$). There was no significant main effect of gender ($F(1, 35) = .87$, $p = .36$), nor a significant interaction between gender and condition ($F(3, 35) = 1.04$, $p = .39$).

To test whether the cue influenced which food participants selected first choices were divided into whether the first item was a sweet or savoury food. A chi-square test showed that condition had no significant effect on whether sweet was chosen first ($\chi^2(3) = 4.67$, $p = .20$). Condition had no significant effect on whether savoury was chosen first ($\chi^2(3) = 6.53$, $p = .088$).

The first food selected was unrelated to which food items were rated as being the most pleasant or being wanted the most (p 's $> .05$).

Table 3.4. The effects of conditioned stimuli on behavioural measurements.

Behavioural Measurement	Test	P value	Significant?
Latency to first food selected	ANOVA	.20	NS
First food selected	Chi-square	.20	NS
Food most selected	Chi-square	.088	NS

Whilst the cue did not have an effect on which food was selected first, the cue may have had a sustained effect over the course of the five minutes. A 5 (Time: Number of food selections in first minute, Number of food selections in second minute, Number of food selections in third minute, Number of food selections in fourth minute, Number of food selections in fifth minute) \times 2 (Food Type: sweet, savoury) by Condition mixed-measures ANOVA revealed a significant main effect of food ($F(1, 37) = 45.77$, $p = .001$, $\eta^2 = .55$), with more selections occurring to savoury foods ($3.90 \pm .30$) than sweet ($1.53 \pm .15$). There was also a significant main effect of time ($F(4, 148) = 10.88$, $p < .001$, $\eta^2 = .23$). Specifically, more selections were made in the first ($3.41 \pm .22$) minute than the second ($2.84 \pm .21$), third ($2.58 \pm .17$), fourth ($2.63 \pm .18$), fifth ($2.12 \pm .22$) (p 's $< .06$). More selections were made in the

second minute compared to the fifth minute ($p = .007$). All other comparisons were not significant (p 's $> .05$). There was a significant interaction between time and condition ($F(12, 148) = 2.06, p = .02, \eta^2 = .14$), however post hoc comparisons revealed no significant condition differences across time (p 's $> .05$). There was a significant interaction between food and time ($F(4, 148) = 3.12, p = .02$). Post hoc tests revealed that whilst the number of sweet selections did not decrease over time ($F(4, 208) = .25, p = .91$), savoury selections did ($F(4, 208) = 7.85, p < .001$). Significantly more savoury selections were made in the first minute ($5.26 \pm .47$) than the third ($3.59 \pm .33$), fourth ($3.40 \pm .34$) and fifth ($2.76 \pm .40$), but not the second ($4.06 \pm .38$) ($p = .09$). More selections were made in the second minute than the fifth ($p = .06$) but all other comparisons were not significant. Also, in each minute more savoury selections were made than sweet (p 's $< .05$).

Whilst there was no significant difference between the number of selections, the increased intake may be due to the *DSchoc* condition taking larger bite sizes. A 4-way (Condition) independent measures ANOVA was performed on average bite size (total grams eaten/total number of selections). It revealed a trend for a main effect of condition ($F(3, 37) = 2.49, p = .076, \eta^2 = .17$). The *No Cue* condition ($6.40 \pm .76$) had larger bite sizes than the *DS-* condition ($3.68 \pm .65$) ($p = .06$), but not significantly different from the *DSchoc* ($4.69 \pm .62$) and *DS crisp* ($4.69 \pm .68$) (p 's $= 1$).

3.4. Discussion

The aim of the experiment was to investigate the effect of discriminative stimuli on intake. Specifically, it was predicted that stimuli associated with the opportunity to win chocolate rewards if participants produced the correct response and stimuli associated with the opportunity to win crisp rewards would increase intake compared to stimuli associated with earning no rewards and when no stimuli are presented. Explicitly, this increase would be specific to the food reward that had the stimulus had been associated with winning (for example, the cue associated with earning chocolate rewards would lead to an increase in chocolate consumption).

The present experiment suggests that a stimulus associated with earning no rewards suppresses intake compared to a stimulus associated with earning chocolate rewards. This suppression of intake was a non-food-specific, general decrease in intake. The suppression of intake of a *DS-* does, somewhat, contradict the findings of previous research showing not a suppression of a *DS-* but a potentiation of the *DS+* (Havermans et al., 2009; Hogarth et al.,

2003). However, to our knowledge there has not been an investigation into the effect of discriminative stimuli on intake (previous operant studies have used cravings measures, for example, Havermans et al., 2009). This, potentially, surprising finding may just be the effect operant-conditioned stimuli has on intake in humans. Also, it is to be noted that a majority of previous research in humans and animals uses simply a CS+ and CS- and without the appropriate control it would be unknown whether any difference found would be due to an increase in behaviour elicited by the CS+ or a suppression of a behaviour elicited by the CS-.

There are a number of reasons why the *DS-* elicited a suppression of intake. It could be suggested that the withholding of responding on either key in the presence of the *DS-* was transferred into the intake test so that people withheld their response in this situation as well. However, people's suppression ratio (a measure of how much people withhold their responding; Annau & Kamin, 1961) was unrelated to the amount they ate ($p > .05$). This renders this explanation unlikely. Another explanation could be found in the differences between the previous Pavlovian studies and this task. In the previous Pavlovian studies (which found a potentiation of feeding in the presence of the CS+) the CS- was a neutral taste. This neutral taste is neither aversive nor rewarding. In comparison, in this study the *DS-* is the absence of winning. In comparison to the other stimuli, which win you chocolate or crisps and thus are highly rewarding, this *DS-* is negative rather than neutral. Although this later explanation could explain the intake finding, it does not explain why there was no differences found in any ratings or time taken to first selection.

Previous research looking into the effect of cue-potentiated feeding have sated participants before conducting the intake test (Weingarten, 1983; Galarce et al., 2007; Reppucci & Petrovich, 2012). Birch, McPhee, Sullivan and Johnson (1989) trained preschool children to associated the presence of one stimulus with the opportunity to eat snack foods. Another stimulus was associated with toys and thus not associated with food. Even whilst sated, children ate significantly more calories in the presence of the food-associated stimulus than in the presence of the non-food-associated stimulus; however, this depended on explicit awareness of the association; those unaware did not eat differently in response to the different stimuli. Additionally these children were shown to respond to the food-associated stimulus by eating within one second of presentation, which was not seen in the presence of the non-food-associated stimulus. Whilst Birch et al (1989) interpret their finding as an increase in intake in the CS+ group, the lack of an appropriate control means their difference may be due to a suppression of intake in the CS-. The effect of these stimuli have been shown to be highly specific. Cornell, Rodin, and Weingarten (1989) fed participants to satiety before

exposing them to a prime (one mouthful) of either pizza or ice-cream or no prime. When given an ad libitum test of pizza and ice cream, the prime enhanced its intake relative to the other-equally preferred food. Being sated or non-sated has been shown to affect attentional bias (Castellanos et al., 2009) as well as implicit wanting and explicit liking (Finlayson, King, & Blundell, 2007b). However, there are neurological (Holland & Gallagher, 2003; McDannald, Saddoris, Gallagher, & Holland, 2005; Hovancik, 1978) and psychological (Holman & Mackintosh, 1981) differences between the stimulus-stimulus (S-S*) associations, reported in the previously mentioned studies, and a stimulus that indicates whether a specific response will obtain a reward (termed a discriminative stimulus [DS]).

To our knowledge, no food research in humans has investigated the effect of a DS on intake whilst sated. The previous experiment was replicated however prior to the intake session participants were satiated on a high-calorie, high-protein milkshake due to its effectiveness as suppressing subsequent appetite (Johnson & Vickers, 1993; Kissileff, 1985; Booth, Chase, & Campbell, 1970; Fryer, Moore, Williams, & Young, 1955; Hill & Blundell, 1986; de Castro & Elmore, 1988).

Experiment 2

3.5. Method

Table 3.5. Method synopsis.

Method	Task
<i>Control of hunger state</i>	Participants refrained from eating for 2 hours.
<i>Conditioning phase</i>	Participants associated stimuli with foods on screen.
<i>Contingency awareness test</i>	Presentation of stimuli and asked which foods were associated with each stimulus.
<i>Preload</i>	Participants consumed milkshake preload.
<i>Intake test</i>	Participants were presented with a selection of food items with the conditioned stimuli on the bowls containing the food/drink.

3.5.1. Design

A between groups (*DS+* condition, *DS-* condition, and *No Cue* condition) design was used to compare the effects of conditioned stimuli on total calories eaten from sweet and

savoury foods in a taste evaluation task. Half of each condition was sated prior to a snack intake test. Latency to first food selected was also measured.

3.5.2. Participants

One hundred and three healthy participants consented to take part in a study entitled 'cognitive performance on sensory evaluations'. However, data from four participants were excluded due to computer error during the conditioning phase and the snack intake test. The ninety-nine remaining participants (50 male) had a mean age of 21.7 years (range 18-37) and BMI of $23.46 \pm .34$ kg/m². The experimental protocol was approved by The University of Sussex's Ethics Committee and conformed with British Psychological Society rules on ethical conduct. Participants gave written informed consent at the start of the test session, and they were compensated by payment of £5 and/or awarded course credits on completion of the study.

Since women with a history of dieting can show an insensitivity to flavour-based learning (Brunstrom, Downes, & Higgs, 2001) and a different response to food cues than unrestrained eaters (Fedoroff, Polivy & Herman, 1997), potential participants completed the Three Factor Eating Questionnaire (Stunkard & Messick, 1985), and were excluded if they scored the average (median) of seven or higher on the Restraint scale. Potential participants with a history of eating disorders, who were diabetic, had specific allergies to any ingredients used in the study, who smoked more than five cigarettes per week and who were on prescription medication (excluding the contraceptive pill) were excluded.

3.5.3. Stimuli

3.5.3.1. Test stimuli. The visual cues were adapted from a series of images constructed by Kuwayama (1973) and subsequently used in a study on cue-based learning in a food context (Laane, Aru, & Dickinson, 2010). To select images that were distinct and neutral, a pilot study was conducted where 12 participants rated the novelty and hedonic appeal of 29 images. Four images rated as both high in novelty and neutral in terms of liking were selected as stimuli for use in this study (see Figure 3.1).

3.5.3.2. Reward stimuli. There were two food stimuli presented, both of which were pictures of common snacks (crisps or chocolate: see Figure 3.1). These images were 8cm x 8cm.

3.5.4. Control of hunger state

Hunger can alter brain activation (LaBar et al., 2001), and these areas have been associated with stimulus salience attribution (Amaral, Price, Pitkänen, & Carmichael, 1992). For this reason, participants were asked to refrain from eating and to drink only water for 2 hours prior to starting the experiment. Similar to previous research (Yeomans, Spetch, & Rogers, 1998) to ensure abstinence participants were informed that a saliva swab may be taken at the start of the session. This swab was never actually taken.

3.5.5. Preload

The preload was given to participants 30 minutes before intake test. The 144g preload consisted of 112g of vanilla ice cream (Carte D'Or) and 132g of semi-skimmed milk. This totalled 300 calories.

3.5.6.1. Procedure

Participants arrived at the laboratory between 12 - 2pm for the start of the test day. Participants indicated their hunger and thirst on separate 100pt line scales from 0 (not at all hunger/ thirst) to 100 (extremely hungry/thirsty) disguised as a mood rating questionnaire, presented using Sussex Ingestive Pattern Monitor (SIPM, version 2.103, University of Sussex). This, and the rest of the experiment, took place in small air-conditioned cubicles within the Psychopharmacology laboratory at the University of Sussex.

3.5.6.2. Operant Conditioning phase. Conditioning took place in small air-conditioned cubicles. Participants were instructed that they would be playing a computer game. The instructions read:

"In this game you may collect points by pressing the 'm' or 'z' key. By pressing these keys you may earn points for crisps or chocolate. You will have 30 seconds per trial. You may earn multiple points per trial. Pay attention! Not every 'z' or 'm' key press will earn you a point. You may have to press more than once. Press SPACE to begin."

After participants had verbally instructed that they understood the instructions, they were left in the room to complete the task. During this task 1 of 4 target images were presented in the centre of the screen for 30 seconds. Above this was written "'press 'z' or 'm'". During this time participants had to press either the 'z' or 'm' key to earn a reward. They were not informed which button was the correct choice. When participants correctly paired the target symbol with the appropriate button press pressed a certain number of times (operating

on a variable-ratio schedule of 5 presses), they were presented with a specific food picture (Table 3.2). These images were presented in the bottom left corner of the screen for two seconds before being removed. During this two second period no key presses were counted. After 30 seconds the stimulus was removed from the screen simply showing a blank screen for 10 seconds. The four stimuli were presented in a random order before repeating in a different random order four more times (20 presentations in total). Two images (*DS-*) were merely presented on-screen for 30 seconds and the pressing of either key produced no reward (called extinction stimuli). The reward and key altered between images. One *DS* signalled a chocolate stimulus if the 'z' key was pressed a certain number of times. One *DS* signalled a crisp stimulus if the 'm' key was pressed a certain number of times. All images, reward and keys were counterbalanced across participants (for example, for one participant Image 1 would signal chocolate if the 'z' key was pressed a certain number of times whereas for another participant Image 2 would have signalled chocolate with if the 'm' key was pressed. The task was custom programmed in E-Prime (version 1.2).

3.5.6.3. Contingency Awareness. Using similar methodology to previous research (Wardle, Mitchell & Lovibond, 2007), participants were presented with each image and asked "Was this picture paired with winning NOTHING/CHOCOLATE/CRISPS most of the time in this experiment?". They rated their answer on a 100pt VAS, end-anchored with 'Definitely Was' (scored 100) and 'Definitely wasn't' (scored 0), programmed using E-Prime. Participants were classified as aware of the *DS-US* contingency if they rated the paired US as more likely to be paired with the paired *DS* than any of the unpaired *US*'. For example, to be aware of the chocolate-paired *DS* a participant would have to give a higher rating for that *DS* on CHOCOLATE scale than the NOTHING or CRISPS scales.

3.5.6.4. Preload. Immediately after completing the contingency awareness test, half of the participants completed 100pt VAS ratings on "How HUNGRY/THIRSTY/FULL/NAUSEOUS do you feel right now?", end-anchored with 'Extremely' (scored 100) and 'Not At All' (scored 0). They then received the milkshake preload. Participants tasted one mouthful of the milkshake and completed VAS ratings on "How THICK/FILLING/PLEASANT/SWEET/FAMILIAR/CREAMY is the milkshake?" end-anchored with 'Extremely' (scored 100) and 'Not At All' (scored 0). They then drank the entire milkshake before completing another set of the HUNGRY/THIRSTY/FULL/NAUSEOUS ratings. They were then instructed to wait in the waiting room for 30 minutes whilst the intake test phase of the experiment was set up in the same room.

3.5.6.5. Testing. At the start of the intake test, participants were instructed that they would now be completing a taste evaluation task. After completing hunger and thirst ratings

disguised as a mood questionnaire using SIPM, participants were informed that they would be conducting a series of ratings on a selection of foods and they would have five minutes during which they must try them all and that they could eat and drink as much as they wanted as any remaining food would have to be thrown away. The experimenter then provided them with four bowls of food (two sweet and two savoury items [see Table 3.2 for nutritional information]) on a tray. After verbally instructing that they had understood the instructions the experimenter brought the test items into the room.

Participants were randomly assigned to one of three groups; *DS+* ($n = 29$), *DS-* Group (*DS-*) ($n = 27$), and *No Cue* Group (*No Cue*) ($n = 25$). For the *DSchoc* Group, the bowls of the test foods were labelled with stickers (4cm x 4cm) of the image associated with winning chocolate. The *DS-* group had one of the two non-winning cues present (There was no difference between which of the two *DS-* was present on intake and so results were collapsed for this group). The *No Cue* group had *No Cue* on the test items.

In the intake test participants were informed that they will be rating these foods and drinks on their visual properties, and not to consume the food. Participants then answered 'how much do you want some of Sample X now?' and 'how pleasant would it be to experience a mouthful of Sample X now?' for each of the samples in a random order. They rated their answer on a 100pt VAS scale, end-anchored with 'Extremely' or '(scored 100)' and 'Not At All' (scored 0). Participants then completed hunger and thirst ratings. Participants completed a series of ratings ('how salty/sweet/familiar/savoury/ pleasant is Sample X?' and 'how much do you want more of Sample X?') after sampling one mouthful of each food on the same VAS scale. After completing these ratings participants were informed that they would have a few minutes to familiarise themselves with the samples and if more was needed to call the experimenter. Once ready participants clicked the screen (this was to control onset in order to measure reaction times). After five minutes they were instructed (using SIPM) to stop eating and to complete another set of hunger and thirst ratings. After completion the remaining food was weighed.

Participants were video-recorded throughout the intake test in order to analyse what type of food they ate first, how quickly they selected the first food and how many selections they made.

Participants were then given a debriefing questionnaire. This involved asking participants to state what they believed to be the purpose of the experiment and how this may have affected them in any way. Their height and weight was then measured followed by payment.

3.5.7. Statistics

Power calculations based on the experiment 1 indicated 92 participants were needed.

The effects of cue-manipulation on overall intake was assessed using independent measures ANOVA with three Conditions (*DS+*, *DS-*, *No Cue*) and whether participants were sated or non-sated (Preload). Since it was expected that men would eat more than would women, gender was also included (Gender: Males, Females). It was predicted that participants in the *DS+* condition would consume more than those in the *DS-* and *No Cue* conditions. Then we tested whether awareness of the stimulus-food association affects intake as in previous research (Birch et al., 1989), a 2 (Contingency Awareness: Aware, Unaware) by Condition independent measures ANOVA was conducted on total grams consumed.

To test whether any overall effects of cue on intake were specific to the relevant cue-associated foods, a 4 (Food type eaten: total grams eaten from chocolate, total grams eaten from crisps, total grams eaten from pretzels, total grams eaten from flapjacks) by Condition, Preload and Gender mixed measures ANOVA was performed. To test whether awareness of the stimulus-food association affects intake, a 4 (Food type eaten: total grams eaten from chocolate, total grams eaten from crisps, total grams eaten from pretzels, total grams eaten from flapjacks) by Condition and Contingency Awareness mixed measures ANOVA was performed.

To test whether the preload sated participants a repeated measures t-test was performed on hunger ratings before and after preload consumption.

To test whether gender, consuming the preload and the cue-manipulation modified liking for the snack foods at test prior to intake, a 4 (Food type: liking rating for chocolate, liking rating for crisps, liking rating for pretzels, liking rating for flapjacks) by Condition, Preload and Gender mixed measures ANOVA. The same analyses were performed for liking ratings post five-minute eating period. Similar analyses were performed for wanting ratings post prior to intake and after the five minute eating period.

To test whether the cue influenced the latency to first food selected a Condition by preload independent measures ANOVA was performed on absolute latency to first food selection.

3.6.1. Results

The critical aspect of the conditioning phase of the procedure was the development of associations between discriminative stimuli and their respective rewards. Eighteen participants did not win any rewards during the conditioning task. Due to this they were not included in any of the following analyses. Eighty-one participants remained; 14 in the Sated *DS+* condition, 15 in the Non-sated *DS+* condition, 14 in the Sated *DS-* condition, 13 in the Non-sated *DS-* condition, 11 in the Sated *No Cue* condition, and 14 in the Non-sated *No Cue* condition. Data was log-transformed where significant Levene's indicated it appropriate. To remove problems with sphericity, significance levels were adjusted using Greenhouse-Geisser or Huynh Feldt where appropriate.

3.6.2. Intake Analysis

There was no significant main effect of condition ($F(2, 69) = .80, p = .454$), no significant main effect of preload ($F(1, 69) = .13, p = .723$), and no significant main effect of gender ($F(1, 69) = 20.53, p < .001, \eta^2 = .23$) with post hoc tests showing males (133.94 ± 6.41) consumed more than females (93.66 ± 6.16). However, there were two outliers (z scores of 4.39 and 3.47) and so these were removed from the dataset. This showed that there was no significant main effect of condition ($F(2, 67) = .22, p = .801$), and no significant main effect of preload ($F(1, 67) = .07, p = .789$). There was a significant main effect of gender ($F(1, 67) = 16.57, p < .001, \eta^2 = .20$), with males (122.52 ± 5.24) eating significantly more than females (93.66 ± 4.78). There was also a significant condition and gender interaction ($F(2, 67) = 4.83, p = .011, \eta^2 = .13$). Post hoc tests showed that whilst there was no significant difference between conditions for males ($F(2, 35) = 1.40, p = .260$), there was a trend for a significant effect for females ($F(2, 38) = 3.17, p = .053, \eta^2 = .14$). Females in the *DS+* condition (107.85 ± 7.54) ate more than females in the *DS-* condition (82.14 ± 7.26) ($p = .056$), but not differently from the *No Cue* condition (89.47 ± 7.26) ($p = .262$). Other conditions did not differ (p 's $> .05$). See Figure 3.4. There was a significant interaction between condition and preload ($F(2, 67) = 3.86, p = .026$). However, post hoc tests showed no difference between conditions when sated ($F(2, 35) = 1.98, p = .153$) or non-sated ($F(2, 38) = .80, p = .459$). There was a significant gender and preload interaction ($F(1, 67) = 4.56, p = .036, \eta^2 = .06$). Whilst males and females did not differ when sated ($t(45) = 1.432, p = .159$), males (129.44 ± 7.23) ate significantly more when non-sated than females (85.54 ± 4.82) ($t(47) = 5.09, p < .001$, effect size $r = .60$). There was no significant three-way interaction ($F(2, 67) = .02, p = .981$).

Due to the effects the outliers had on the results, the outliers will not be included in any of the following analyses. When participants who had correctly guessed the true purpose of the experiment were removed from the analysis similar results were obtained, and so these participants will remain in the analyses.

When analysed in terms of calories consumed similar results were obtained; no significant main effect of condition ($F(2, 67) = .22, p = .801$), no significant main effect of preload ($F(1, 67) = .07, p = .789$), with a significant interaction between the two ($F(2, 67) = 3.86, p = .026, \eta^2 = .10$). There was a significant main effect of gender ($F(1, 67) = 16.57, p < .001, \eta^2 = .20$), and a significant interaction between condition and gender ($F(2, 67) = 4.83, p = .011, \eta^2 = .13$). There was a significant preload by gender interaction ($F(1, 67) = 4.56, p = .036, \eta^2 = .06$). There was no significant group, preload and gender interaction ($F(2, 67) = .19, p = .981$).

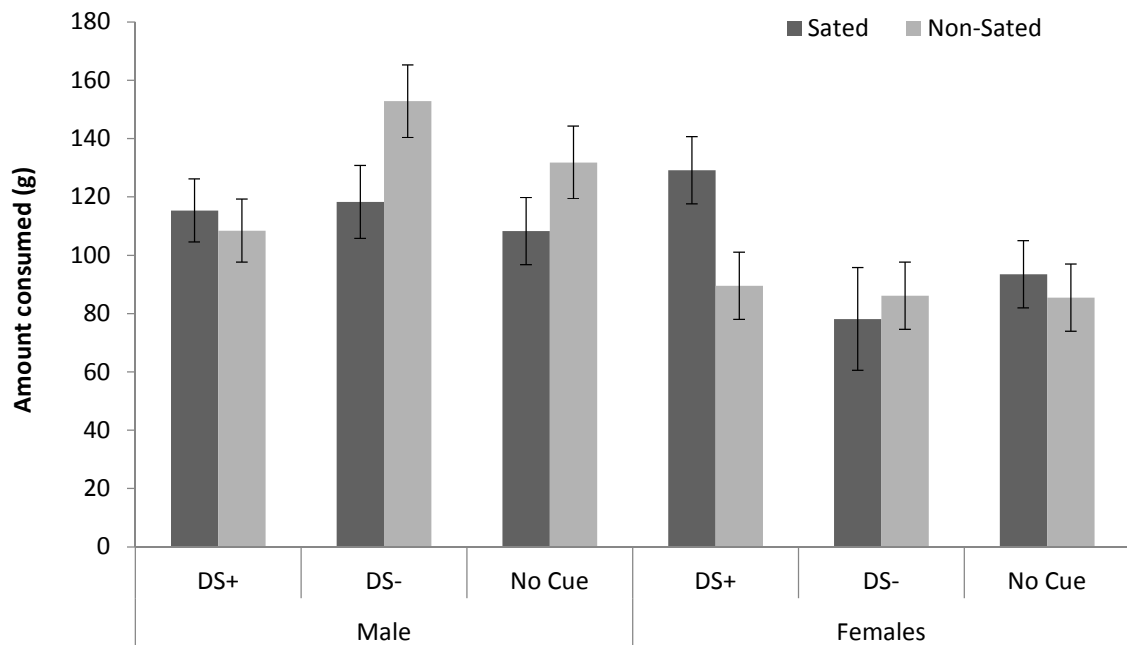


Figure 3.4. Amount consumed (g) in the three cue conditions split by gender. Data are mean \pm 1 SEM.

DS+ male sated $n = 8$, DS+ female sated $n = 6$, DS+ male non-sated $n = 8$, DS+ female non-sated $n = 7$, DS- male sated $n = 7$, DS- female sated $n = 7$, DS- male non-sated $n = 6$, DS- female non-sated $n = 7$, No Cue male sated $n = 3$, No Cue female sated $n = 7$, No Cue male non-sated $n = 6$, No Cue female non-sated $n = 7$.

3.6.3. Intake Analysis with contingency Awareness

Preload and Gender were not included in the following analysis due to small number of unaware participants. There was no significant main effect of condition ($F(1, 52) = .63, p = .430$), no significant main effect of contingency awareness ($F(1, 52) = 1.32, p = .257$), with no significant interaction between the two ($F(1, 52) = .24, p = .627$). The same results were obtained when analysed by total calories eaten ($p's > .05$).

3.6.4. Type of food consumed

There was a significant main effect of Food Type ($F(2.89, 193.70) = 76.42, p < .001, \eta^2 = .53$). Post Hoc tests showed that flapjacks (43.27 ± 2.20) were eaten more than chocolate (32.64 ± 2.00), crisps ($19.84 \pm .95$) and pretzels ($12.43 \pm .86$) ($p's \leq .001$). Chocolate was eaten more than crisps and pretzels ($p's < .001$). Crisps were eaten more than pretzels ($p < .001$). There was no significant difference between the amount eaten by the different conditions ($F(2, 67) = .21, p = .810$). There was no significant interaction between Food Type and Condition ($F(5.78, 193.70) = 1.43, p = .206$). See Figure 3.5.

There was no significant main effect of Preload ($F(1, 67) = .07, p = .799$). There was no significant interaction between Preload and Food Type ($F(2.89, 193.70) = 1.59, p = .194$). There was no significant interaction between Food Type, condition and Preload ($F(5.78, 193.70) = 1.37, p = .233$). There was a significant interaction between condition and Preload ($F(2, 67) = 3.69, p = .030, \eta^2 = .10$), however post hoc tests revealed no significant differences between the conditions when sated ($F(2, 35) = 2.00, p = .15$), nor non-sated ($F(2, 38) = .78, p = .47$).

There was a significant difference between males and females ($F(1, 67) = 16.26, p < .001, \eta^2 = .20$). Specifically, males (30.62 ± 1.31) ate more than females (23.47 ± 1.20) on average grams eaten of the foods. There was a significant interaction between Food Type and Gender ($F(2.89, 193.70) = 3.21, p = .026, \eta^2 = .05$). Post Hoc tests showed that males ate more grams of chocolate, pretzels and flapjacks than females ($p's > .05$). Males and females did not differ in the amount eaten of crisps ($t(94) = 1.33, p = .185$, effect size $r = .14$). There was a significant interaction between Condition and Gender ($F(2, 67) = 4.81, p = .011$). There was a significant interaction between Preload and Gender ($F(1, 67) = 3.69, p = .030, \eta^2 = .06$). There was a significant interaction between Food Type, Condition and Gender ($F(5.78, 193.70) = 3.36, p = .004, \eta^2 = .09$), as shown in Figure 3.5. Split by gender, there was a significant effect of food for both genders ($p's > .05$). There was no significant effect of condition for males ($F(2, 35) = 1.07, p = .36$), but was a significant effect for females ($F(2, 38) = 3.36, p = .046, \eta^2 = .15$). Also, whilst there was no significant interaction between Food Type and Condition for males ($F(5.57, 89.04) = 1.54, p = .18$), there was a significant interaction for females ($F(5.18, 95.91) = 3.40, p$

= .007, $\eta^2 = .15$). Post hoc tests showed that females in the DS+ condition (38 ± 5.64) ate significantly more chocolate than the DS- condition (17.79 ± 2.52) ($t(16.64) = 3.27$, $p = .005$, effect size $r = .63$) and the *No Cue* condition, ($21.38, 2.97$) ($t(24) = 2.61$, $p = .02$, effect size $r = .47$). There were no other significant comparisons (p 's > .05).

There was no significant interaction between Food Type, Preload and Gender ($F(2.89, 193.70) = 1.47$, $p = .226$). There was no significant interaction between Condition, Preload and Gender ($F(2, 67) = .01$, $p = .988$). There was a significant interaction between Food Type, Group, Preload and Gender ($F(5.78, 193.70) = 2.39$, $p = .032$, $\eta^2 = .07$), however, there were limited number of people in certain categories (only three males in the sated *No Cue* condition) and so limited meaningful conclusions could be drawn.

When analysed in terms of calories consumed the same results were obtained.

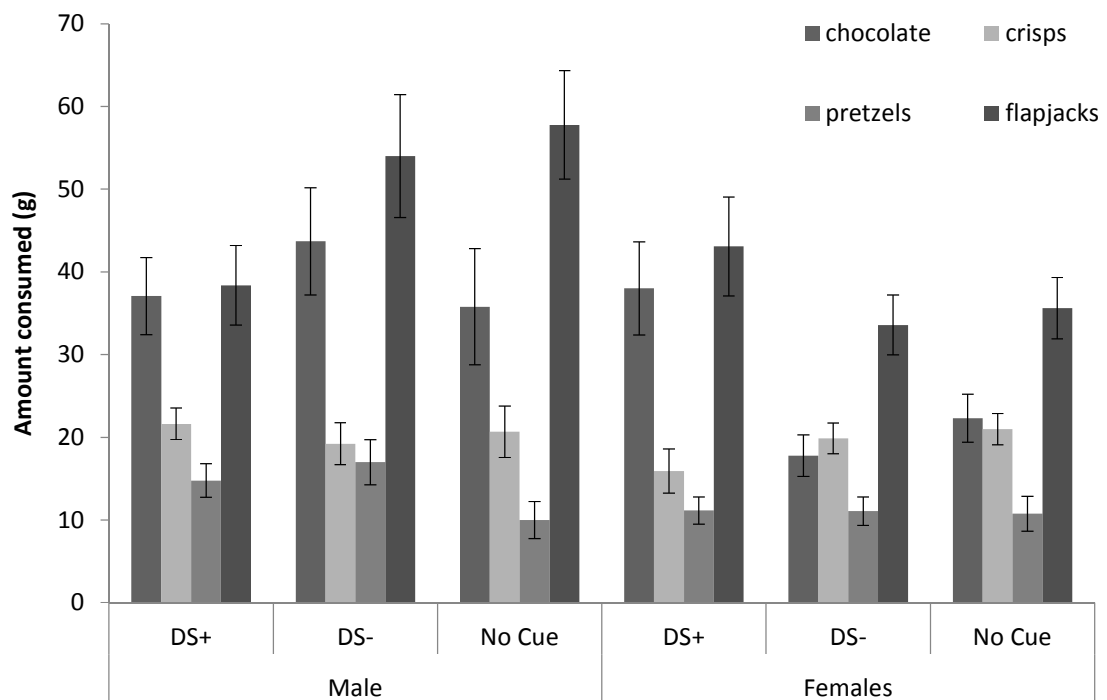


Figure 3.5. Amount consumed (g) of the different food items in the three cue conditions split by gender. Data are mean \pm 1 SEM. DS+ male $n = 16$, DS+ female $n = 13$, DS- male $n = 13$, DS- female $n = 14$, No Cue male $n = 9$, No Cue female $n = 14$.

3.6.5. Type of Food Eaten by Contingency Awareness.

There was no significant main effect of Contingency Awareness ($F(1, 52) = 1.30$, $p = .260$) no significant main effect of condition ($F(1, 52) = .67$, $p = .416$) nor a significant

interaction between the two ($F(1, 52) = .27, p = .606$). There was no significant interaction between type of food eaten and contingency awareness ($F(2.57, 133.42) = 1.60, p = .198$). There was no significant interaction between type of food eaten and condition ($F(2.57, 133.42) = 1.28, p = .283$). There was no significant interaction between type of food eaten, condition and contingency awareness ($F(2.57, 133.42) = .38, p = .738$).

3.6.6. Preload manipulation

Participants were significantly less hungry after the preload (47 ± 4) than before (72 ± 3) ($t(46) = 7.82, p < .001$, effect size $r = .76$). There was no significant effect of condition on hunger ratings before preload or after preload (p 's $> .05$).

3.6.7. Participant characteristics

There was no significant between conditions on BMI, age, restraint or disinhibition (p 's $> .05$). There was no significant difference between conditions in appetite ratings before conditioning ($F(2, 76) = .08, p = .920$). There were no significant differences between conditions in regards to total rewards won during the operant conditioning task, not when split by number of chocolate or crisp rewards won (p 's $> .05$).

To test whether conditions differed in subjective ratings of appetite a Condition by Preload independent measures ANOVA was performed on appetite ratings. There was no significant difference in appetite ratings between the groups before being presented with the food in the intake test ($F(2, 73) = .43, p = .655$). Before seeing the food, there was a significant main effect of preload ($F(1, 73) = 6.46, p = .013, \eta^2 = .08$), with non-sated participants (69 ± 3) rating themselves as hungrier than sated participants (58 ± 3). There was no significant interaction between group and preload ($F(2, 73) = .24, p = .786$).

There was no significant difference in appetite ratings between the groups after being presented with the food in the intake test ($F(2, 73) = .97, p = .383$). There was a trend for a difference between sated and non-sated participants ($F(1, 73) = 3.79, p = .056, \eta^2 = .06$), with non-sated participants (79 ± 3) rated as hungrier than sated (72 ± 3). There was no significant interaction between group and preload ($F(2, 73) = .02, p = .982$).

3.6.8. Conditioning

To check whether the number of stimulus-reward associations affected intake a correlation was performed between number of rewards won and total grams consumed. The amount of grams consumed in the intake test was significantly correlated with total number of

rewards won ($r(94) = .22, p = .032$), but not separately with number of chocolate rewards won ($r(93) = .17, p = .102$). Total chocolate rewards won was uncorrelated with total grams of chocolate consumed ($r(93) = .12, p = .232$). There was no significant difference between the conditions on number of chocolate or crisp rewards won ($p's > .05$).

To see if there were behavioural differences between aware and unaware participants a t-test was performed on total number of presses in response to the different stimuli. There was no significant difference between aware and unaware participants on total number of presses to the chocolate stimuli ($t(85) = 1.03, p = .305$), and crisp stimuli ($t(85) = .38, p = .704$). There was a significant difference between aware and unaware participants on total number of presses for both DS- stimuli ($p's > .05$), with higher presses for aware participants for both DS- stimuli (265.32 ± 34.80 and 274.79 ± 34.64) than unaware participants (148.67 ± 39.10 and 141.81 ± 43.08).

3.6.9. Hedonic and sensory evaluations of the test snacks

3.6.9.1. Expected Liking Prior to Intake.

There was a significant main effect of liking ratings ($F(3, 201) = 11.92, p < .001, \eta^2 = .15$). Post-hoc tests showed that chocolate (82 ± 2) was rated as significantly more pleasant than crisps (73 ± 2), pretzels (64 ± 3) and flapjacks (73 ± 3) ($p's < .05$). Crisps were liked more than pretzels ($p = .025$) and equal to flapjacks ($p = 1$). Pretzels were liked less than flapjacks ($p = .013$).

There was no significant main effect of condition ($F(2, 67) = 1.88, p = .160$), nor a significant main effect of gender ($F(1, 67) = .22, p = .645$). There was no significant interaction between condition and gender ($F(2, 67) = 1.02, p = .368$). There was a significant effect of preload ($F(1, 67) = 4.75, p = .033, \eta^2 = .07$), with the non-sated group (76 ± 2) rating the foods as significantly more pleasant than the sated group (70 ± 2).

There were no significant interactions between liking ratings of each food and condition ($F(6, 201) = .91, p = .487$), or liking ratings and gender ($F(3, 201) = .44, p = .722$), or liking ratings and preload ($F(3, 201) = 1.87, p = .136$). There was no significant interaction between condition and preload ($F(2, 67) = 1.94, p = .152$). There was no significant interaction between gender and preload ($F(1, 67) = 1.46, p = .232$). There was no significant interaction between liking ratings, condition and preload ($F(6, 201) = .33, p = .921$). There was no significant interaction between liking ratings, gender and preload ($F(3, 201) = .23, p = .877$). There was no significant interaction between condition, gender and preload ($F(2, 67) = .02, p = .980$).

There was no significant interaction between the pleasantness ratings of the different foods, conditions and gender ($F(6, 201) = 1.89, p = .084$). The main ANOVA revealed no significant four-way interaction between liking ratings, condition, gender and preload ($F(6, 201) = 1.83, p = .094$).

3.6.9.2. Expected Wanting Prior to Intake.

A mixed measures ANOVA revealed a significant difference in the wanting ratings for the different foods ($F(3, 201) = 10.85, p < .001, \eta^2 = .14$). Post-hoc tests showed that chocolate (80 ± 2) was wanted significantly more than flapjacks (72 ± 2) and pretzels (63 ± 3) and crisps (p 's $< .05$). Crisps were higher than pretzels ($p = .021$) but not different from flapjacks ($p = 1$). Pretzels were significantly lower than flapjacks ($p = .049$).

There was no significant main effect of condition ($F(2, 67) = .42, p = .656$), nor a significant main effect of gender ($F(2, 67) = 2.19, p = .144$). There was no significant interaction between condition and gender ($F(1, 67) = 1.81, p = .171$). There was a significant main effect of preload ($F(1, 67) = 7.97, p = .006$), with the sated group (67 ± 2) wanting the foods significantly less than the non-sated group (75 ± 2). There was no significant interaction between condition and preload ($F(2, 67) = .96, p = .387$), nor a significant interaction between gender and preload ($F(1, 67) = 2.35, p = .130$). There was no significant interaction between the wanting ratings of the different foods and condition ($F(6, 201) = .69, p = .659$). There was no significant interaction between wanting ratings and gender ($F(3, 201) = 2.04, p = .110$). There was no significant interaction between wanting ratings and preload ($F(3, 201) = 1.30, p = .275$). There was no significant interaction between wanting ratings, condition and preload ($F(6, 201) = .28, p = .946$). There was no significant interaction between wanting ratings, gender and preload ($F(3, 201) = .45, p = .717$). There was no significant interaction between condition, gender and preload ($F(2, 67) = .5, p = .866$).

There was a significant interaction between wanting ratings for the different foods, condition and gender ($F(6, 201) = 2.21, p = .044, \eta^2 = .06$). Post-hoc tests revealed that females in the DS+ condition (62 ± 5) wanted the crisps significantly less than females in the *No Cue* condition (85 ± 4) ($t(24) = 3.45, p = .002$, effect size $r = .58$), and the DS- condition (81 ± 4) ($t(25) = 2.91, p = .008$, effect size $r = .50$). The DS- and *No Cue* conditions did not differ (p 's $> .05$). The conditions did not significantly differ on wanting ratings of any other food items (p 's $> .05$). For males the conditions did not significantly differ on any foods (p 's $> .05$).

There was no significant four-way interaction between the wanting ratings of the different foods, condition, Gender and Preload ($F(6, 201) = 1.81, p = .099$).

3.6.9.3. *Explicit Liking.*

A mixed measures ANOVA revealed a significant main effect of liking ratings ($F(3, 201) = 13.03, p < .001, \eta^2 = .16$). Crisps (83 ± 2) were liked significantly more than pretzels (64 ± 3) and flapjacks (71 ± 3) (p 's $< .05$) but the same as chocolate (80 ± 3) ($p = 1$). Chocolate was liked more than pretzels and flapjacks (p 's $< .05$). Pretzels and flapjacks did not differ ($p = .338$).

There was no significant difference in liking ratings between conditions ($F(2, 67) = .99, p = .377$), or between males and females ($F(1, 67) = .17, p = .683$). There was no significant interaction between condition and gender ($F(2, 67) = .20, p = .822$). There was no significant interaction between the liking ratings of the different foods and condition ($F(6, 201) = 1.16, p = .327$). There was no significant interaction between liking ratings and preload ($F(3, 201) = .90, p = .443$). There was no significant interaction between liking ratings, condition and gender ($F(6, 201) = 1.02, p = .412$).

There was a significant effect of preload on liking ratings ($F(1, 67) = 4.40, p = .040, \eta^2 = .06$). Across foods, the non-sated group (78 ± 2) liked the foods significantly more than the sated group (72 ± 2) ($p = .054$). There was no significant condition by preload interaction ($F(2, 67) = .11, p = .900$), nor a significant gender and preload interaction ($F(1, 67) = .73, p = .398$). There was no significant interaction between the liking ratings of the different foods, condition and preload ($F(6, 201) = .70, p = .648$). There was no significant interaction between liking ratings, gender and preload ($F(3, 201) = .13, p = .942$). There was no significant interaction between condition, gender and preload ($F(2, 67) = .26, p = .775$). There was no significant four-way interaction between liking ratings, condition, gender and preload ($F(6, 201) = .81, p = .563$).

3.6.9.4. *Explicit Wanting.*

A mixed measures ANOVA revealed a significant difference in the wanting ratings for the different foods ($F(3, 201) = 13.55, p < .001, \eta^2 = .17$). Specifically, Crisps (73 ± 3) were wanted more than chocolate (56 ± 4), pretzels (51 ± 3) and flapjacks (53 ± 3) ($p \leq .001$). The other foods did not differ from each other (p 's $> .05$).

There was no significant main effect of condition ($F(2, 67) = .64, p = .530$), or a significant main effect of gender ($F(1, 67) = .86, p = .357$). There was no significant interaction between wanting ratings and condition ($F(6, 201) = .76, p = .602$), or with gender ($F(3, 201) = .27, p = .849$). There was no significant interaction between condition and gender ($F(2, 67) = .10, p = .908$). There was no significant interaction between wanting ratings for the different foods, condition and gender ($F(6, 201) = .37, p = .901$).

There was a significant main effect of preload ($F(1, 67) = 9.66, p = .003, \eta^2 = .13$), with the non-sated (64 ± 3) group wanting the foods more than the sated group (53 ± 3). There was

no significant interaction between wanting and preload ($F(3, 201) = 2.35, p = .074$). There was no significant condition by preload interaction ($F(2, 67) = .38, p = .685$). There was a significant interaction between gender and preload ($F(1, 67) = 6.66, p = .012, \eta^2 = .09$). A post-hoc ANOVA revealed that there was no significant difference in wanting ratings between males and females when sated ($F(1, 35) = 1.09, p = .304$), however there was a significant difference when non-sated ($F(1, 36) = 6.69, p = .014, \eta^2 = .14$), with males (70 ± 4) wanting the foods significantly more than females (58 ± 3). The main ANOVA revealed there was no significant interaction between the wanting ratings for the different foods, condition and preload ($F(6, 201) = .41, p = .873$). There was no significant interaction between ratings, gender and preload ($F(3, 201) = .33, p = .802$). There was no significant interaction between condition, gender and preload ($F(2, 67) = .94, p = .429$). There was no significant four-way interaction between the wanting ratings for the different foods, condition, gender and preload ($F(6, 201) = 1.15, p = .336$).

3.6.9.5. Sweetness.

Given that odours which have been paired with sweet tastes subsequently smell sweeter (Stevenson, Prescott & Boakes, 1995), the hypothesis that the presence of the chocolate associated cue would enhance the perceived sweetness of the snacks in the *DS+* condition was also tested using a 4 (Food type: sweetness rating for chocolate, sweetness rating for crisps, sweetness rating for pretzels, sweetness rating for flapjacks) by Condition, Preload and Gender mixed measures ANOVA. This revealed a significant difference in the sweetness ratings for the different foods ($F(2.03, 135.92) = 381.83, p < .001, \eta^2 = .85$). Chocolate (89 ± 2) was rated as significantly sweeter than flapjacks (77 ± 2), crisps (13 ± 2), and pretzels (16 ± 2) (p 's $< .001$). Flapjacks were sweeter than pretzels and crisps (p 's $< .001$). Pretzels and crisps were not significantly different ($p = .691$).

There was a significant main effect of condition on sweetness ratings across foods ($F(2, 67) = 3.18, p = .048, \eta^2 = .09$). The *DS+* condition (52 ± 2) rated the foods as significantly sweeter than the *No Cue* condition (45 ± 2) ($p = .043$), but not different to the *DS-* (49 ± 2) ($p = 1$). The *DS-* and *No Cue* did not differ ($p = .337$). There was no significant interaction between the sweetness ratings of the different foods and condition ($F(4.06, 135.92) = .25, p = .910$).

There was no significant main effect of gender on sweetness ratings ($F(1, 67) = .40, p = .529$). However, males and females significantly differed on the sweetness ratings of different foods ($F(2.03, 135.92) = 3.25, p = .041, \eta^2 = .05$). Post-hoc tests showed that males and females did not significantly differ on their ratings of chocolate, pretzels and crisps (p 's $> .05$). However, females (82 ± 2) rated the flapjacks as significantly sweeter than males

(71 ± 4) did ($t(49.528) = 2.28, p = .027$, effect size $r = .31$). There was no significant interaction between condition and gender ($F(2, 67) = 2.70, p = .074$), nor a significant interaction between the sweetness ratings of the different foods, condition and gender ($F(4.06, 135.92) = 1.73, p = .146$).

There was no significant main effect of preload ($F(1, 67) = 1.01, p = .318$), nor a significant interaction between the different sweetness ratings and preload ($F(2.03, 135.92) = .35, p = .709$). There was no significant interaction between condition and preload ($F(2, 67) = 1.00, p = .37$). There was no significant condition, gender and preload interaction ($F(2, 67) = .44, p = .646$).

There was a significant interaction between gender and preload ($F(1, 67) = 9.68, p = .003, \eta^2 = .13$). Split by preload, a post-hoc ANOVA revealed that there was trend for a significant difference between males and females both when sated ($F(1, 35) = 3.74, p = .061, \eta^2 = .11$), and when non-sated ($F(1, 36) = 4.37, p = .044, \eta^2 = .11$). However, when sated males (52 ± 2) rated the foods as significantly sweeter than the females' (47 ± 2) ratings, whereas when non-sated the females (52 ± 2) had higher sweetness ratings than the males (44 ± 3).

There was no significant interaction between the sweetness ratings of the different foods, condition and preload ($F(4.06, 135.92) = .35, p = .850$). There was no significant interaction between the different foods' sweetness ratings, gender and preload ($F(2.03, 135.92) = 1.40, p = .251$). There was no significant four-way interaction between the sweetness ratings, condition, gender and preload ($F(4.06, 135.92) = .97, p = .428$).

3.6.10. Behavioural analysis

Previous research (Birch et al., 1989) has indicated that conditioned stimuli elicit rapid conditioned responses to be produced. To investigate whether this happened to the discriminative stimuli the latency to first food selected, first food selected and number of selections made were coded by the experimenter from the video-playback of each eating session, with 10% of the videos also being coded by a second experimenter to check reliability. Cohen's K revealed very strong agreement between experimenters ($k = 0.92, SE = 0.07$). Contingency awareness had no effect on any of the following outcome measures ($p's > .05$) and so was not included in any of the following analyses.

An independent measures ANOVA revealed no significant main effect of condition ($F(2, 63) = 1.09, p = .34$), preload ($F(1, 63) = .27, p = .60$), nor a significant interaction between the two ($F(2, 63) = 1.20, p = .31$). See Figure 3.6.

To test whether the cue influenced which food participants selected first choices were divided into whether the first item was a sweet or savoury food. A chi-square test showed that

condition had no significant effect on which food was first selected (sweet or savoury) for sated ($X^2(2) = 1.21, p = .55$) or non-sated participants ($X^2(2) = 1.01, p = .60$). Condition also had no significant effect on which food was selected the most for sated ($X^2(2) = 1.40, p = .50$), and non-sated participants ($X^2(2) = 2.28, p = .32$).

Which food (sweet or savoury) participants rated as most pleasant before tasting the food was associated with which food they chose first for sated participants ($X^2(1) = 6.86, p = .01$). For non-sated participants there was no significant association ($X^2(2) = 1.13, p = .29$). After tasting one mouthful of the different foods pleasantness ratings were not significantly associated with which food was selected first for sated ($X^2(1) = 1.37, p = .24$), and non-sated participants ($X^2(1) = .41, p = .52$).

Which food (sweet or savoury) participants rated as most wanted before tasting the food was not significantly associated with which food they chose first for sated participants ($X^2(1) = 1.11, p = .29$). For non-sated participants there was no significant association ($X^2(2) = 2.45, p = .12$). After tasting one mouthful of the different foods wanting ratings were significantly associated with which food was selected first for sated ($X^2(1) = 4.55, p = .03$), but not non-sated participants ($X^2(1) = 2.29, p = .13$).

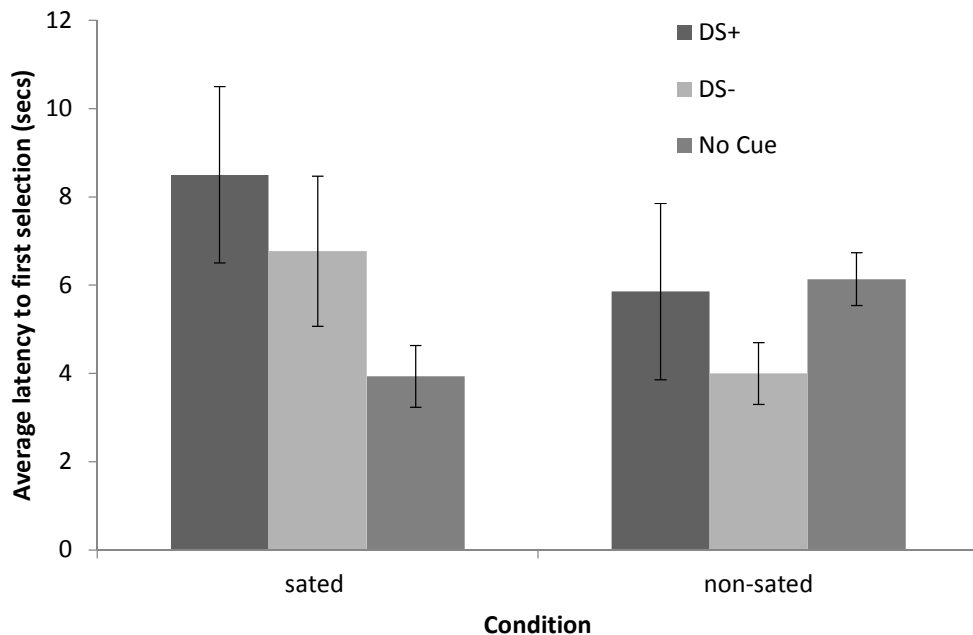


Figure 3.6. Latency to first food selected in the three cue conditions when sated and non-sated. Data are mean \pm 1 SEM. DS+ sated $n = 14$, DS+ non-sated $n = 15$, DS- sated $n = 14$, DS- non-sated $n = 13$, No Cue sated $n = 10$, No Cue non-sated $n = 13$.

Whilst the cue did not have a significant effect on which food was selected first, the cue may have had a sustained effect over the course of the five minutes. A 5 (Time: Number of selections in the first minute, Number of selections in the second minute, Number of selections in the third minute, Number of selections in the fourth minute, Number of selections in the fifth minute) \times 2 (Food type: sweet, savoury) by Condition and Preload mixed measures ANOVA revealed, as before, a significant main effect of time ($F(4, 252) = 12.87, p < .001, \eta^2 = .17$), and a significant interaction between time and preload status ($F(4, 252) = 2.81, p = .03, \eta^2 = .03$). See Table 3.6.

There was a significant main effect of food type ($F(1, 63) = 115.43, p < .001, \eta^2 = .64$), with more savoury selections ($3.91 \pm .20$) being made than sweet ($1.40 \pm .10$) across all minutes. There was also a significant interaction between food and condition ($F(2, 63) = 4.03, p = .02, \eta^2 = .10$). Post hoc tests revealed that, across minutes, there was a trend for the *No Cue* condition to make more savoury selections on average than the *DS+* ($t(42) = 1.95, p = .06$, effect size $r = .29$), and *DS-* condition ($t(45) = 1.83, p = .07$, effect size $r = .26$). There was no significant difference between *DS+* and *DS-* conditions on savoury foods, (p 's $> .05$), and no conditions differed on average number of sweet selections (p 's $> .05$).

All other main effects and interactions were not significant (p 's $> .05$).

A Condition by Preload independent measures ANOVA was performed average bite size (total grams eaten/total number of selections). It revealed a significant main effect of condition ($F(2, 63) = 3.43, p = .04, \eta^2 = .08$). Post hoc tests showed that the *DS+* condition ($5.31 \pm .46$) had significantly larger average bite sizes than the *No Cue* condition ($3.62 \pm .47$) ($p = .05$) but not different from the *DS-* condition ($4.36 \pm .43$) ($p = .49$), which itself did not differ from the *No Cue* condition ($p = .61$). There was no significant main effect of preload ($F(1, 63) = 2.99, p = .09$), or no significant interaction between condition and preload ($F(2, 63) = .84, p = .44$).

Table 3.6. Number of sweet and savoury food selections by the three cue conditions sated and non-sated across the five minute intake test.

Preload	Group	Min 1 Sweet	Min 1 Savoury	Min 2 Sweet	Min 2 Savoury	Min 3 Sweet	Min 3 Savoury	Min 4 Sweet	Min 4 Savoury	Min 5 sweet	Min 5 savoury
Sated	<i>DS+</i>	2.30 (.58)	4.40 (.73)	1.90 (.48)	3.90 (.84)	2.00 (.49)	2.70 (.40)	1.30 (.40)	3.40 (.83)	1.80 (.47)	3.00 (.60)
	<i>DS-</i>	1.50 (.29)	4.86 (.48)	1.29 (.32)	3.93 (.75)	1.14 (.33)	4.07 (.73)	1.00 (.28)	3.79 (.70)	.93 (.30)	3.79 (.66)
	<i>No Cue</i>	1.56 (.34)	4.67 (.78)	.44 (.18)	6.56 (.87)	1.44 (.44)	4.22 (.83)	.67 (.33)	6.78 (.91)	1.11 (.35)	4.00 (.97)
	<i>DS+</i>	1.75 (.48)	5.17 (1.59)	1.92 (.42)	2.92 (.80)	1.08 (.50)	3.83 (1.02)	1.17 (.46)	2.58 (.61)	.75 (.28)	2.92 (.94)
Non-sated	<i>DS-</i>	2.18 (.64)	4.00 (.62)	1.45 (.28)	3.64 (.78)	1.91 (.46)	3.00 (.82)	1.73 (.45)	2.09 (.51)	1.27 (.41)	3.18 (.74)
	<i>No Cue</i>	1.85 (.39)	4.38 (.94)	1.23 (.30)	4.54 (.83)	1.08 (.38)	4.85 (.61)	1.46 (.29)	2.92 (.57)	.92 (.24)	3.31 (.57)

3.7. General Discussion

The aim of the experiment was to investigate the effect of discriminative stimuli on intake whilst sated. Specifically, it was predicted that the stimuli associated with earning no rewards (*DS-*) would suppress intake compared the stimulus associated with obtaining a chocolate reward (*DS+*).

The present experiment did not replicate the findings of Experiment One by finding no significant difference between conditions in intake whilst non-sated. In fact, when non-sated the results indicated that the *DS+* group actually ate the least in comparison to the *DS-* and *No Cue* groups. When sated the *DS+* did eat more than the other two groups however the differences were not significant. Additionally, whilst there was *No Cue*-potentiation there was also no evidence for cue-specificity; the *DS+* did not influence people to eat more chocolate compared to other foods or compared to other groups. This does partially support the finding from Experiment one.

One potential explanation for the lack of replication in Experiment two could be due to a gender difference in cue-reactivity; a finding not previously found in Experiment one. In Experiment two, whilst there was *No Cue*-potentiation for males in response to the discriminative stimuli, females in the *DS+* condition ate more than the *DS-* condition, as predicted in the hypothesis. Similar to findings from previous chapters, this cue-potentiation was specific to the reward it was previously conditioned with; females in the *DS+* condition ate more chocolate than the other conditions, whilst similar amounts of the other foods were

eaten. Whilst previous research has found gender differences in cue reactivity in regards to drugs (Field & Duka, 2004; Robbins, Ehrman, Childress, & O'Brien, 1999) and trained inhibition of hunger in response to food (Wang et al., 2009), to our knowledge no studies have found this effect in regards to cue-potentiated feeding. In Experiment two males and females did not differ on variables previously shown to affect cue-reactivity, such as BMI (Jansen et al., 2003) or restraint (Fedoroff, Polivy, & Herman, 1997). Although there was no significant interaction between group, gender and preload in the amount eaten, there does appear to be a stronger cued-potential effect for females whilst sated compared to when non-sated. The potential susceptibility of females to cue-potentiated feeding whilst sated could be an important contributor to higher body fat percentages shown in women compared to men (Gallagher et al., 1996), however, more research is needed to support this novel and interesting finding. However, there remains an important distinction between the findings of Experiment one and Experiment two. Just looking at the results for females in Experiment two suggests an cue-potential whilst Experiment one suggests a cue-suppression of intake. The reason for this discrepancy is unknown.

The satiating nature of the preload used in Experiment two must be questioned. Whilst the preload decreased hunger ratings, upon presentation of the test foods hunger ratings for the sated condition increased dramatically (almost to the same level as non-sated individuals). Whilst an increase in hunger in response to presentation of hedonic snack foods is to be expected, the high subjective hunger ratings may have meant that the preload was not as satiating as should have been. Previously research have fed animals (Weingarten, 1983) and humans (Birch et al., 1989) to satiation, whereas the present experiment used a standardised quantity. This discrepancy could be responsible for the lack of a cue-potentiated feeding effect in the sated condition, however this does not explain the lack of cue-potential in the non-sated condition.

One possibly surprisingly finding from both experiments was the lack of an effect of contingency awareness on amount of food consumed. Although this contrasts with previous research (Birch et al., 1989) this does support the findings from previous chapters and other research (Brunstrom, 2004) indicating a lack of necessity for awareness in specialist systems, such as the gustatory system. However, it is to be noted that the methodology used in these experiments differed significantly from those used in chapter two. It is interesting that a highly cognitive task, such as this, in which a stimulus is associated with a hypothetical reward, found no effect of contingency awareness on intake. Whilst it is acknowledged that the method of

measuring contingency awareness used in this experiment may not have measured it accurately, the finding does support previous research from our laboratory.

In both experiments the discriminative stimuli did not have an effect on which food was selected first, nor how fast food was selected. Whilst this contradicts previous research (Birch et al., 1989), this does support the findings from chapter two. This suggests that implicit 'wanting' (measured by preference tests [Berridge & Robinson, 1998]) does not have a role in which food was selected first. Interestingly, in Experiment two explicit 'wanting' and 'liking' ratings were related to the first food item selected. Prior to tasting one mouthful of the food items participants' liking ratings, but not their wanting ratings, predicted which food they would select first, however, after tasting one mouthful of the foods, the reverse was true; first food selected was predicted by wanting and not liking scores. This may be due to the hedonic value of a reward (liking) being determined by past experience which is re-evaluated post experience with the reward (Balleine & Dickinson, 1991), whereas according to the Bindra-Toates model 'wanting' (incentive salience) incorporate new information regardless of experiencing the hedonic value of the reward. However, due to the nature of the questions used it may be the fact that the liking ratings post experience with the foods is based on the past experience on the mouthful just eaten whereas the wanting ratings are predictive of your future behaviour. The latter may mean people adjust their behaviour to coincide with their ratings (Festinger, 1962).

As discussed in the introduction, there are substantial differences between stimulus-stimulus associations and a stimulus indicative of when a response-stimulus association can occur (Holland & Gallagher, 2003; Holman & Mackintosh, 1981). Whilst the methodology used in these experiments has produced contradictory findings, it remains a novel method of investigating the effect of discriminative stimuli of ingestive behaviour. However, to our knowledge there has been no direct comparison of Pavlovian and discriminative stimuli associated with food in humans, which warrants future investigation.

In conclusion, the current experiments, whilst producing contrasting results, provide a useful method of investigating the effect of discriminative stimuli on consumption. A potential gender difference in cue-reactivity also deserves further investigation.

Chapter 4: Brain regions underlying neural responses to Pavlovian and Discriminative stimuli.

4.1. Introduction

In humans, neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), have been consistently used to view changes in neural activity in response to food. Commonly in fMRI investigations of appetitive behaviour images of food are presented to participants and changes in brain responses are measured. For example, Simmons, Martin and Barsalou (2005) presented pictures of appetizing foods (compared to locations) to participants in an MRI scanner. They found that pictures of food activated the right insula/operculum and the left orbital frontal cortex (OFC) (both gustatory areas). However, images of locations are substantially different from images of specific items. When participants viewed food compared to visually matched non-food images, activation was found in the similar regions such as the left OFC and insular/opercular cortex (Porubská, Veit, Preissl, Fritsche, & Birbaumer, 2006). Similar studies have been conducted altering physiological drive state. For example, Führer, Zysset and Stumvoll (2007) showed participants pictures of foods or non-foods after 14 hours or one hour of fasting. In response to food images, regardless of hunger state, enhanced brain activity was observed in areas previously associated with reward such as the insula, the left striate and extra-striate cortex, and the anterior mid prefrontal cortex (PFC). When participants were fasted they had greater stimulation in the left anterior cingulate cortex, superior occipital sulcus and the right amygdala in response to food images compared to when they had fasted for only one hour. Similar results have been found after a 24hr and 2 hour fasting period (Uher, Treasure, Heining, Brammer, & Campbell, 2006).

However, 'food' images is a broad category, and previous research has shown that certain characteristics of the foods presented in the images can have differential effects. Killgore et al. (2003) showed that whilst high and low-calorie foods collectively activated the amygdala and ventromedial PFC, they differed in their abilities to activate other regions. For example, the high-calorie foods activated areas of the PFC, thalamus, hypothalamus and cerebellum, whereas the low-calorie foods activated the medial OFC, gustatory cortex and various temporal regions. The insular cortex has also been shown to be activated by high-calorie foods (Frank et al., 2010). The distinct patterns of regional activations based on the caloric characteristics of the displayed food categories has been shown to be dependent on

fasting state (Goldstone et al., 2009) and BMI (Stoeckel et al., 2008; Stoeckel et al., 2009). Similar findings have been found using fattening and non-fattening foods (Schur et al., 2009).

However, the visual experience of food is different from orally experiencing food. Frank et al. (2003) gave participants an oral experience of either sucrose or artificial saliva. Sucrose selectively increased activation in the OFC and temporal cortical regions compared to when they tasted artificial saliva. However, as previous research has shown, sweet solutions can be perceived hedonically different dependent on liker status (Looy & Weingarten, 1992; Yeomans et al., 2006, Yeomans, Mobini, Bertenshaw, & Gould, 2009; Yeomans, Prescott, & Gould, 2009). Rudenga and Small (2013) gave participants individually calibrated weak and strong sucrose solutions (which half rated as pleasant and half as unpleasant). When the strong sucrose solution was rated as pleasant participants showed greater activation in the ventromedial PFC compared to when the strong sucrose was rated as unpleasant. Using quinine as an unpleasant taste Small et al. (2003) contrasted quinine with sucrose. They found that the cerebellum, pons, middle insula, and amygdala responded to intensity of the stimulus regardless of pleasantness. In comparison, the anterior insula/operculum (extending to the OFC) responded in a valence-specific manner. The right caudolateral OFC responded preferentially to pleasant over unpleasant tastes, whereas the left dorsal anterior insula/opercular region responded in the opposite manner.

In regards to more complex tastes, such as milkshakes, Babbs et al. (2013) gave participants milkshake or tasteless solutions in an MRI scanner. They found a negative association between BMI and caudate nucleus activation in response to the milkshake.

Behavioural experiments have shown that cues associated with food can potentiate consumption when eating occurs in its presence (Birch et al., 1989; Cornell, Rodin & Weingarten, 1989; Fedoroff, Polivy, & Herman, 1997). Whilst research (Holland, 1990) has shown that cues can substitute for the food/taste itself, evidence from fMRI studies has shown that cues associated with basic tastes, such as sweet taste, activate different brain regions compared to the tastes themselves. In one study (O'Doherty, Deichmann, Critchley, & Dolan, 2002) participants were presented with one of three arbitrary visual stimuli whilst in the scanner. Shortly after stimulus presentation either a pleasant taste (a glucose solution), an aversive taste (a salty solution) or a neutral control taste (artificial saliva) was delivered. The conditioned stimuli signalling the pleasant taste produced activation in the dopaminergic midbrain, posterior dorsal amygdala, striatum and OFC. However, only the OFC was activated

by actual receipt of the pleasant taste. This highlights the distinction between neural activity in response to food-associated cues and food-receipt.

In regard to complex tastes, Burger and Stice (2014) conducted a study in which geometric shapes predicted the delivery of either a palatable milkshake or a tasteless solution. Exposure to the cues predicting the milkshake produced activation in the caudate which increased over repeated exposures to the cues (indicating *in vivo* conditioning). During receipt of the milkshake a decrease in putamen and ventral pallidum activation was observed, possibly due to food reward habituation.

However, Pavlovian conditioned stimuli (CS) (i.e. stimuli that are passively associated with an outcome) produce neurologically (McDannald, Saddoris, Gallagher, & Holland, 2005) and psychologically (Chait et al., 1988) different responses from discriminative stimuli (DS) (i.e. stimuli that indicate when a response will be associated with obtaining an outcome; Black, Osborne, & Ristow, 1977). O'Doherty et al. (2004) showed that stimulus-response-outcome associations (DS) produce distinct neural activation compared to stimulus-outcome associations (CS). Specifically, arbitrary stimuli associated with juice if the correct response was performed activated the dorsal striatum, whereas arbitrary stimuli passively associated with juice activated the ventral striatum.

Previous behavioural findings in our laboratory have suggested that Pavlovian (CS) and discriminative stimuli (DS) can substantially affect intake. Pavlovian stimuli (CS) associated with a sweet taste can potentiate feeding whereas a discriminative stimulus (DS) associated with the opportunity to work for a reward produces a different effect. Specifically, a stimulus associated with not obtaining a chocolate reward, suppresses intake compared to a stimulus associated with obtaining a chocolate reward.

This study investigated whether cue/food-outcome associations that have been formed through Pavlovian or Instrumental conditioning, elicit differential brain responses. Additionally, individual differences, such as restraint (Fedoroff, Polivy, & Herman, 1997) and weight status (Nisbett, 1968), which have been shown to affect cue-potentiated consumption, were also explored. Participants completed two conditioning tasks before being presented with the stimuli they had learnt in these tasks in an MRI scanner. Firstly, participants completed a discriminative-stimulus task. In this they had the opportunity to associate one DS with a sweet reward (chocolate) if the correct response was produced, whereas another DS with a savoury reward (crisp) if the correct response was produced. Other DS predicted nothing. Secondly, in a separate conditioning task participants were given the opportunity to

experience a sweet or a neutral taste in the presence of novel, visual images using a triangle-disguised training method (Stevenson, Prescott, & Boakes, 1995; Yeomans, Mobini, Elliman, Walker, & Stevenson, 2006). Participants then completed a contingency awareness test in which the stimuli from both conditioning tasks were presented and participants asked which outcome was associated with each. All participants were placed in the MRI scanner, in which they were presented with the stimuli from each of the two conditioning tasks disguised as an oddball task.

It was predicted that discriminative and Pavlovian stimuli will activate neural regions associated with reward, such as the striatum, insula, amygdala, and putamen. It was also predicted that the stimuli associated with a sweet taste or food outcomes would produce activation in the orbitofrontal cortex (OFC), thalamus, and hippocampus compared to stimuli not associated with reward or with a neutral taste.

4.2. Method

4.2.1. Design

A within participants' design contrasted the effects of novel visual cues on neurological responses following prior association of these cues with food rewards. Five conditions were tested: one where cues were associated with the taste of 10% sucrose solution (*CS+*), another cue that had been paired with a neutral taste (*CS-*), another cue associated with the opportunity to win chocolate if the correct response was produced (*DSchoc*), another cue associated with the opportunity to win crisps if the correct response was produced (*DScrisp*), and another cue not associated with obtaining rewards (*DS-*).

4.2.2. Participants.

Seventeen right-handed participants initially consented to participate in a study described as "Brain Regions Underlying Neural Responses to Rewards". Data from one of these were excluded due to showing structural abnormalities. The sixteen (eight male) remaining participants had a mean age of 24 (range 18-47) and BMI of 23.5 ± 0.8 .

Participants gave written informed consent at the start of the test session, and the protocol was approved by The Brighton and Sussex Medical School's Research Governance and Ethics Committee and conformed with British Psychological Society rules on ethical conduct. Participants were compensated by payment of £15 on completion of the study.

Because a history of dieting may decrease sensitivity in flavour-based learning (Brunstrom, Downes, & Higgs, 2001) and alter responses to food cues (relative to unrestrained eaters) (Fedoroff, Polivy & Herman, 1997), participants first completed the Three Factor Eating Questionnaire (Stunkard & Messick, 1985): participants scoring seven or higher on the Restraint scale were not invited to take part in the study. Participants with a history of eating disorders, who were diabetic, had specific allergies to any ingredients used in the study, who smoked more than five cigarettes per week or who presently used prescription medication (excluding the contraceptive pill) were excluded. They were, also, excluded if they had an allergy or aversion to any of the ingredients used in the experiment (such as dairy products, tomatoes, crisps, etc.) stipulated in the information sheet.

4.2.3. Taste Screening Session

One of the key associations in this study was between a novel visual cue and a liked sweet taste (10% sucrose solution). Past research has showed that individual differences in 'sweet-liking' (Looy & Weingarten, 1992) may influence performance in sucrose-based associative learning (Yeomans et al., 2006, Yeomans et al., 2009). To distinguish sweet-likers from sweet-dislikers, participants attended a short taste session at least one day before the main test session. During this session, they were given two samples containing 10% sucrose solution, and two samples of water, to taste, and were then asked to rate each sample for pleasantness, sourness, sweetness, bitterness and saltiness using visual analogue scales ranging from 0 ("Very Unpleasant" or "Not at all") to 100 ("Very Pleasant" or "Extremely"). The label for the dimension evaluated was written above the centre of each line. Ratings were made using Sussex Ingestive Pattern Monitoring (SIPM, version 2.013, University of Sussex). Ten millilitre samples of each solution were refrigerated and brought to room temperature prior to tasting. Participants were defined as sweet-likers if their average rating of the two sucrose solutions was at least 55pt on both the pleasantness and sweetness scale. This procedure was based on previous studies of sweet liking (Yeomans, Prescott & Gould, 2009; Looy & Weingarten, 1992).

4.2.4. Stimuli

4.2.4.1. Conditioned stimuli. The visual cues were adapted from a series of images constructed by Kuwayama (1973) and previously used in cue-based food learning (Laane, Aru, & Dickinson, 2010). To select images that were distinct and neutral in terms of liking, a pilot study was conducted where 12 participants rated the novelty and hedonic appeal of 29 images. Eight images rated as both high in novelty and neutral in terms of liking were selected as

stimuli for use in this study. Which stimuli were used as CS and DS were counterbalanced across participants.

4.2.4.2. Pavlovian reward stimuli. There were four sets of food-reward stimuli, each with three 150ml liquid samples presented in 200ml cups. Within sets A and B the solutions in each sample were the same. In set A (the sweet set) the solutions were 10% sucrose. In set B (the neutral set) the solutions were “artificial saliva” [which consisted of deionised water, 1.865g/l of potassium chloride (KCl), and 0.210g/l of sodium bicarbonate (NaHCO_3) as used by Zampini, Sanabria, Phillips, & Spence (2007)]. In set C (additional set) two solutions were weakly Açai (10 drops per 250g water [Givaudan©]) and one was strongly Açai (35 drops per 250g water [Givaudan©]). In set D (additional set) two solutions tasted of Grapefruit (30 drops per 250g water [Givaudan©]) and one was water.

4.2.4.3. Operant reward stimuli. There were two food stimuli presented, both of which were pictures of common snacks (crisps or chocolate: see Figure 4.1). These images were 8cm x 8cm.

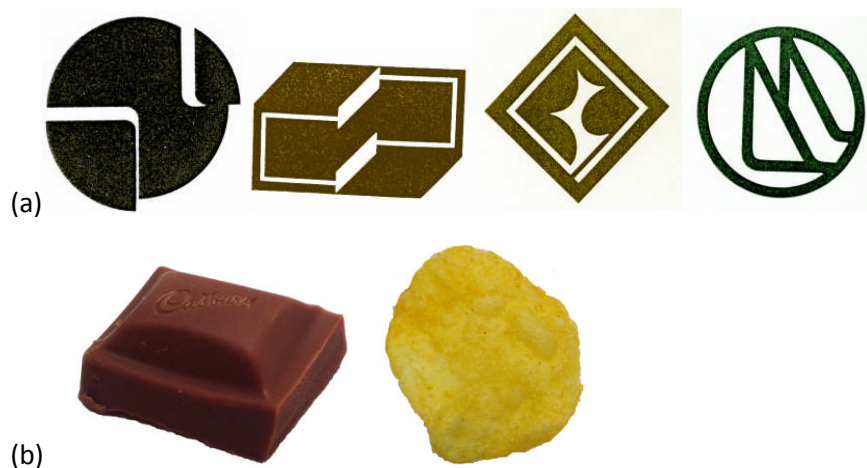


Figure 4.1. The four target discriminative stimuli (a) and two food images (b) used during the operant training phase

4.2.5. Control of hunger state

Hunger can alter brain activation (LaBar et al., 2001) in areas associated with stimulus salience attribution (Amaral, Price, Pitkänen, & Carmichael, 1992). For this reason participants were given a 554kcal standard lunch which consisted of a cheese and cucumber sandwich (40g of cheddar cheese, 30g of cucumber and two slices of white bread [Kingsmill Great Every Day]),

a 34.5g packet of crisps (ready salted, Walkers brand), five cherry tomatoes and a glass of water.

4.2.6. fMRI acquisition.

Images were acquired using a Siemens Avanto 1.5 T MRI scanner at the Clinical Imaging Sciences Centre (CISC) at the University of Sussex.

Whole-brain structural images were acquired using a standard T1 weighted pulse sequence (1mm³ resolution) (MPRAGE_WIDER_GRAPPA). Structural images consisted of a sagittal T1-weights localizer, which was followed by a T1-weighted acquisition of the whole brain in the axial plane (FOV = 256 x 256 mm², 256 x 256 matrix, thickness = 1.0 mm).

Blood-oxygen level-dependent (BOLD) sensitive, gradient-echo T2*-weighted scans were acquired using echo-planar imaging (EPI) comprising of 36 contiguous axial slices (-30 deg. to AC – PC axis; interleaved). The following imaging parameters were used: TR = 3300 ms, TE = 50 ms, acquisition matrix = 64 x 64, in-plane resolution = 3 x 3mm, FoV read = 192 mm, filter = none, field of view = 192 x 192 mm.

A gradient field map using double-echo FLASH sequence was recorded to be used for distortion correction of the EPI images.

4.2.7. Pilot.

Sixteen participants (different participants from those included in the main analysis) completed the discriminative and Pavlovian conditioning tasks before completing the contingency awareness test (see below). Three participants were excluded due to not winning during the operant task. On average participants remembered 74% of the stimuli (SE = 8.77). The mode was 6/6 stimuli recalled and the median was 5/6.

4.2.8. Procedure.

4.2.8.1. Hunger ratings. Participants who had passed the sweet-liker assessment arrived at the laboratory between 1200-1400 for the start of the test day, and consumed the standard lunch. They then were allowed to leave the laboratory for two hours but were instructed to refrain from eating and to only consume water during this time. On returning participants indicated their hunger and thirst on separate 100pt line scales from 0 (not at all hunger/ thirst) to 100 (extremely hungry/thirsty) disguised as a mood rating questionnaire, presented using SIPM. These questions were integrated into a larger mood questionnaire measuring ‘How energetic/calm/clearheaded/strong is your desire to eat/much do you think you could eat/full/tired/headachy do you feel right now?’ on separate 100pt scales from 0 (not

all all) to 100 (extremely). All testing was conducted in small air-conditioned cubicles at the University of Sussex.

4.2.8.2. Pavlovian conditioning phase. The Pavlovian conditioning phase used the triangle-test disguised training method (Stevenson, Prescott, & Boakes, 1995; Yeomans et al., 2006). There were five blocks in total, with four trials (i.e. one presentation of set A, B, C, and D) in each block. During each trial, participants were presented with three solutions. Using E-Prime (version 1.2) participants were instructed the following:

“For this task, you will be presented with an odd-one-out task. You will be asked to take one of the Sets of drinks and place it in front of you. Your task will be to taste each solution and determine which solution is the odd-one-out based on its taste. Some trials will be hard and some will be easy. You must select the solution that you think is the odd-one-out by its location on the tray. For example, if you think the solution on the left of the tray is the odd-one-out then click the picture on the left of the screen. When you try a solution put it back on the tray. Do not pick up the next solution until you have returned the first solution to the tray. Spit the solution into the bottle provided then swill your mouth with water. Please only take one sip of each solution per trial.”

The would-be conditioned stimuli associated with each set were presented in two locations; first using 4 x 4 cm stickers directly placed on the cups and secondly on a 17” LED screen located on a desk in front of the participant (the three images presented equidistance along the screen with the question centred above them). The three cups in each set had the same CS sticker on the side of it which was specific to the solution in the cup (i.e. one CS for sweet solutions, one CS for neutral solutions, etc.). All three CS’ on screen and the cups were the same image. Participants were choosing which CS to pick based on its location corresponding to the samples on the tray. Participants tasted the three samples and once they identified an odd sample had to click on one of the three CS’ on the screen.

To ensure participants understood the task a practice trial (one solution orange and mango squash and two water) was conducted with the experimenter present. For sets A and B the three solutions were identical. This forced participants to closely attend to the flavour of the solutions and also to the stimuli presented on the screen. Sets C and D were control stimuli, used to disguise the purpose of the study. Participants were instructed to only take one sip from each of the three cups per trial as this would ensure a standard amount of CS-US pairings for all participants. After each US sampling, participants were instructed to spit the solution

and rinse their mouth with distilled water. Once participants had completed all four trials participants completed four more sessions (total of 20 trials).

All images, tastes and order of tasting was counterbalanced and randomised across participants.

4.2.8.3. Discriminative Conditioning phase. Participants were told that they would be playing a computer game. The task was programmed using E-Prime (version 1.2). Participants were instructed the following:

“In this game you may collect points by pressing the ‘m’ or ‘z’ key. By pressing these keys you may earn points for crisps or chocolate. You will have 30 seconds per trial. You may earn multiple points per trial. Pay attention! Not every ‘z’ or ‘m’ key press will earn you a point. You may have to press more than once. Press SPACE to begin.”

After participants had verbally instructed that they understood the instructions, they were left in the room to complete the task. During this task one of four target images were presented in the centre of the screen for 30 seconds. Above this was written “‘press ‘z’ or ‘m’”. During this time participants had to press either the ‘z’ or ‘m’ key to earn a reward. They were not informed which button was the correct choice. When participants correctly paired the target symbol (see Figure 4.1) with the appropriate button press pressed a certain number of times (operating on a variable-ratio schedule of 5 presses), they were presented with a specific food picture (see Figure 4.1). These food stimuli were presented in the bottom left corner of the screen for two seconds before being removed. During this two second period no key presses were counted. After 30 seconds the discriminative stimuli was removed from the screen simply showing a blank screen for 10 seconds. The four DS’ were presented in a random order before repeating in a different random order four more times (20 presentations in total). Two images (DS-) were merely presented on-screen for 30 seconds and the pressing of either key produced no reward (called extinction stimuli). One DS signalled a chocolate stimulus if the ‘z’ key was pressed a certain number of times (*DSchoc*). One DS signalled a crisp stimulus if the ‘m’ key was pressed a certain number of times (*DScrisp*). All images, reward and keys were counterbalanced across participants (for example, for one participant Image 1 would signal chocolate if the ‘z’ key was pressed a certain number of times whereas for another participant Image 2 would have signalled chocolate with if the ‘m’ key was pressed. All images and rewarded response was counterbalanced and randomised across participants.

4.2.8.4. Contingency Awareness. Using similar methodology to previous research (Wardle, Mitchell & Lovibond, 2007), participants were presented with each image and asked

“Was this picture paired with WINNING NOTHING/WINNING CHOCOLATE/WINNING CRISPS/A LIQUID WITH A SWEET FLAVOUR/A LIQUID WITH NO FLAVOUR/A LIQUID WITH A SALTY FLAVOUR most of the time in this experiment?”. They rated their answer on a 100pt VAS, end-anchored with ‘Definitely Was’ (scored 100) and ‘Definitely wasn’t’ (scored 0), programmed using E-Prime. Participants were classified as aware of the contingency between the stimulus and the reward if they rated the paired reward stimuli as more likely to be paired with the paired stimulus than any of the unpaired reward stimuli. For example, to be aware of the chocolate-paired DS a participant would have to give a higher rating for that DS on CHOCOLATE scale than the NOTHING or CRISPS scales.

Participants were then instructed that there would be a 30 minute break. During this time the participant and experimenter walked to the Clinical Imaging Science Centre (CISC) at the University of Sussex. The participant then completed a CISC consent form (Including giving GP details). After this they completed a practice of the task they would be completing inside the scanner. The practice task included the same target picture (see below for details) but all other pictures were novel.

4.2.8.5. Within-scanner procedure. The procedure started with a 20 second localiser scan followed by a six minute structural scan. Participants then started the procedure. The procedure was an oddball (event-related) paradigm consisting of 210 trials, divided into two runs of 105 trials. Participants were instructed that they would be looking for a target stimulus (the same target as the practice task). They were informed that when they saw this picture they needed to press the button with their index finger in their right hand. The main task then began. On screen participants saw a fixation cross for, on average, four seconds (jittered between two and six seconds). They then saw a stimulus for two seconds. There were seven types of stimuli each shown semi-randomly (no more than three presentations of the same stimulus in a row) a total of 25 times each. The seven cues were; *DSchoc*, *DScrisp*, *DS-*, *CS+*, *CS-*, a novel stimulus, and the target stimulus. There were also 35 null events (where, after the fixation cross appeared, the fixation cross would be presented for a further 2 seconds) (see Figure 4.2). After 105 trials participants were informed that there would be a 30 second break before continuing the task. To reduce system noise, during this break period the scanner was stopped to minimise the effects of scanner drift. In total, the task lasted 21 minutes and 30 seconds. Finally, a field map was taken.

Participants were then given a debriefing questionnaire. This involved asking participants to state what they believed to be the purpose of the experiment and how this may have affected them in any way. Their height and weight was then measured followed by payment.

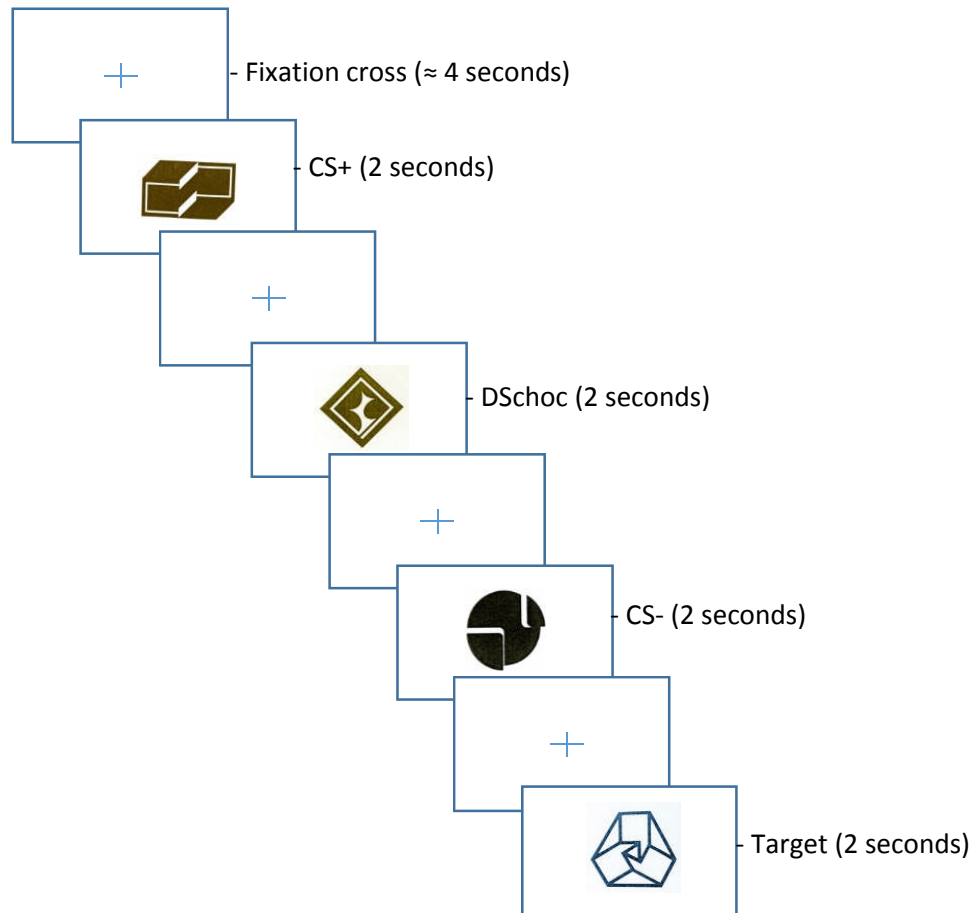


Figure 4.2. Within-scanner procedure example.

4.2.9. fMRI analyses

Power calculations, based on previous chapters and O'Doherty et al. (2004), indicated 16 participants were needed.

Event-related design blood oxygen level dependent (BOLD) responses were analysed using a General Linear Model on a voxel by voxel basis as implemented in SPM8 (Wellcome Dept. of Imaging Neuroscience, London). The data were high-pass filtered (1/128 Hz) to remove low frequency drifts. Data was analysed using SPM8. To allow for steady state magnetization, the first five EPI volumes before the task started were collected but not included in the following analysis.

There were two steps to this procedure. Firstly, in the single subject SPM models responses to all stimuli from both halves of the task (before and after break period) were modelled as events and convolved with the canonical hemodynamic response function. To account for the variance caused by head movement, realignment parameters were also

included as regressors in the model. Five mm FWHM Gaussian smoothing was used to reduce noise.

At the second-level analysis, stimuli were then contrasted using ANOVAs to localise group maxima before performing t-tests to ascertain the direction of the contrast. All p-values are family error-wise corrected. Approximate AR(1) autocorrelation model estimated at omnibus F -significant voxels ($p < .001$), used globally over the whole brain.

Seven regions of interest (ROIs; striatum, Orbitofrontal cortex (OFC), hippocampus, thalamus, putamen, insula, amygdala) associated with stimuli presentation in the fMRI experiment were created with the Wake Forest University (WFU) Pickatlas toolbox (Maldjian, Laurienti, Kraft, & Burdette, 2003). The location of the activated voxels were verified using the WFU Pickatlas toolbox. All stereotactic coordinates are presented in MNI (Montreal Neurological Institute) space.

4.3. Results

4.3.1. fMRI analysis

4.3.1.1. Discriminative Stimuli.

Initially, the individual discriminative stimuli were investigated to ascertain the brain regions that produced activity in response to the stimuli's presentation. The three discriminative stimuli were compared to baseline to ascertain unique regional activity. The *DSchoc* bilaterally activated the hippocampus ($p = .07$). The right striatum and right thalamus were both activated in response to the *DSchoc* (p 's $< .05$). The *DS-* did not produce significant activation in any of the regions of interest (p 's $> .05$) (Figure 4.3).

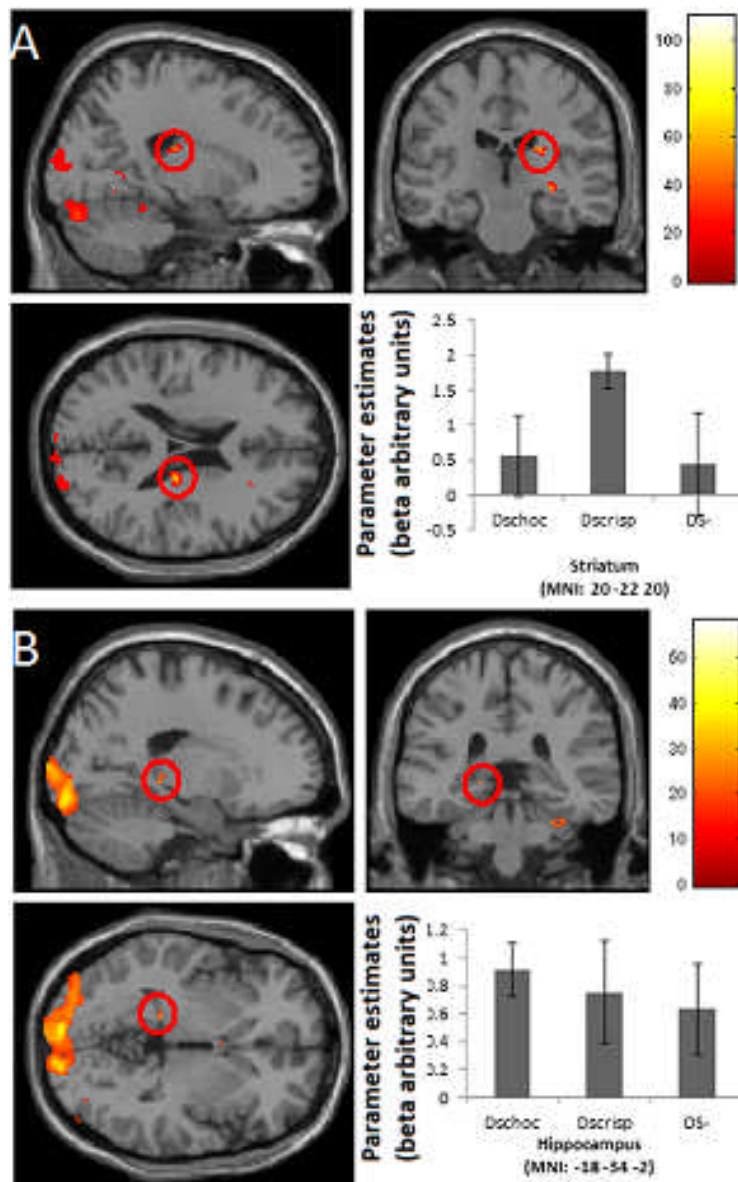


Figure 4.3. Brain activation to discriminative stimuli. **A**, Area of the striatum showing increased activation for *DScrip* presentation. **B**, Area of the hippocampus showing increased activation for *DSchoc* presentation. Colour bars indicate *F* values. Data are presented in mean \pm SEM. MNI, Montreal Neurological Institute.

4.3.1.2. Discriminative stimuli comparisons.

Brain responses to the different rewarding discriminative stimuli were revealed through t-test contrasts of *DSchoc* and *DScrip*. However, this revealed no unique regional activity between the two (p 's $> .05$). *DSchoc* was then contrasted, using t-tests (*DSchoc* $>$ *DS-*), with *DS-* to reveal the brain regions underlying an appetitive discriminative stimulus compared to a non-rewarding discriminative stimulus. This revealed greater activation in the left hemisphere of the striatum ($p = .048$) in response to *DSchoc* than to *DS-*. Another t-test (*DS-*

>*DSchoc*) contrast revealed that the *DS-* produced greater bilateral activation in the orbitofrontal cortex (OFC) ($p = .069$) compared to the *DSchoc*. It also produced greater activation in the thalamus ($p = .08$) in the left hemisphere compared to the *DSchoc*. *DScrisp* was contrasted, using t-tests, with *DS-* similarly (*DScrisp*>*DS-*). The *DScrisp* produced greater activation in the para-hippocampal region in the left hemisphere ($p = .075$) compared to the *DS-*. Another t-test (*DS->DScrisp*) however showed that the *DS-* produced greater bilateral activation in the OFC ($p = .001$) compared to the *DScrisp*.

The appetitive discriminative stimuli (*DSchoc*, *DScrisp*) were then collapsed and contrasted using t-tests with the non-rewarding discriminative stimulus (*DS-*). The first t-test (rewarding discriminative stimuli>*DS-*) showed that the rewarding discriminative stimuli activated the striatum in the left hemisphere ($p = .046$) significantly more than to non-rewarding discriminative stimuli. In contrast (*DS->rewarding discriminative stimuli*), the non-rewarding stimulus bilaterally activated the OFC ($p < .001$) more than the rewarding stimuli.

4.3.1.3. Pavlovian Conditioned Stimuli.

Similar to the discriminative stimuli, the individual Pavlovian stimuli were compared to baseline to ascertain the unique regions activated. The Pavlovian *CS+* produced bilateral deactivation in reward circuitry, such as the striatum ($p < .001$) (Figure 4.4) and the putamen ($p < .001$) (Figure 4.4). Additionally, it produced deactivation in the insula in the left hemisphere ($p = .053$) (Figure 4.4). The Pavlovian *CS-* produced deactivations in the left amygdala ($p = .027$) and left thalamus ($p = .072$) (Figure 4.4).

When contrasted using t-tests, there were no unique regional activity between the Pavlovian *CS+* and the Pavlovian *CS-* (p 's > .05).

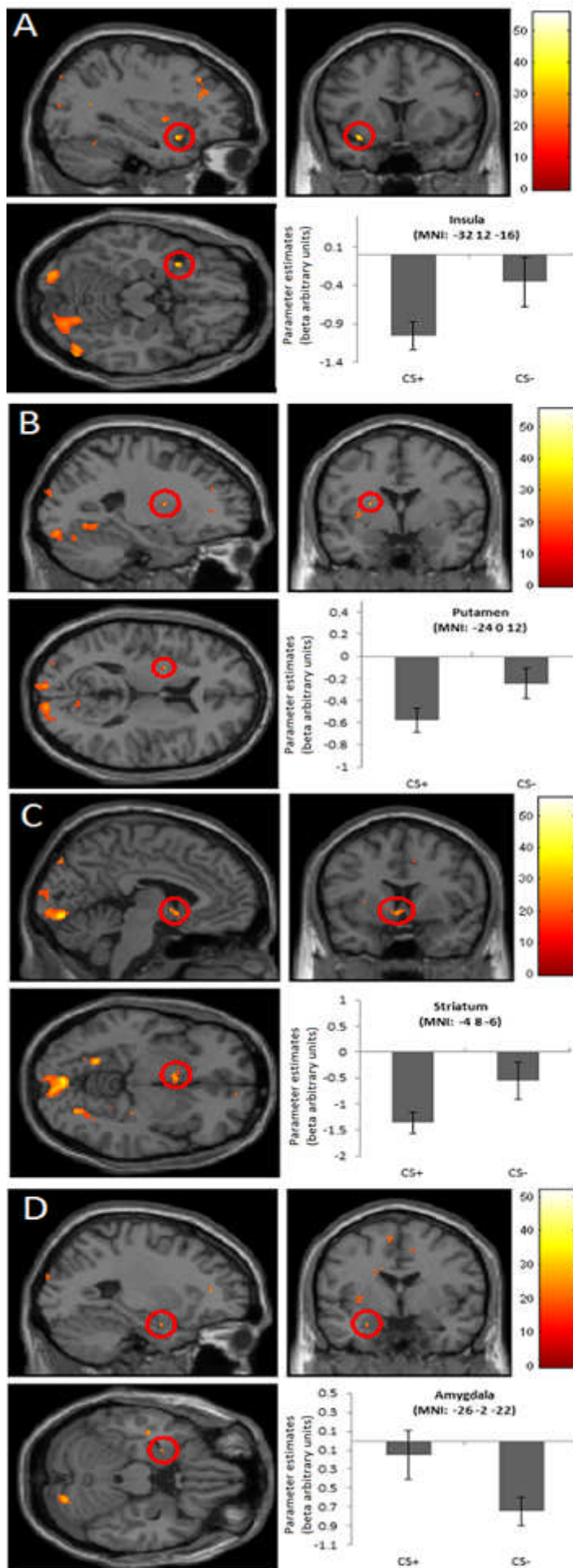


Figure 4.4. Brain activation to Pavlovian stimuli. **A**, Area of the insula showing increased deactivation for CS+ presentation. **B**, Area of the putamen showing increased deactivation for CS+ presentation. **C**, Area of the striatum showing increased deactivation for CS+ presentation. **D**, Area of the amygdala showing increased deactivation for CS- presentation. Colour bars indicate F values. Data are presented in mean \pm SEM. MNI, Montreal Neurological Institute.

4.3.1.4. Comparison of Pavlovian and Discriminative stimuli.

To test what brain regions produced differential neural responses to discriminative and Pavlovian stimuli, the discriminative stimuli (*DSchoc*, *DScrisp* and *DS-*) were collapsed and compared to the collapsed Pavlovian stimuli (*CS+* and *CS-*). T-tests (Discriminative stimuli > Pavlovian stimuli) showed that discriminative stimuli (regardless of associated reward) produced greater activation in areas associated with reward, such as the striatum ($p < .001$) and putamen ($p = .005$) in both hemispheres, and the left insula ($p = .001$). It also produced bilateral greater activation in the amygdala ($p < .001$) and hippocampus ($p < .001$). There were no significant regions that were more activated to Pavlovian stimuli than to discriminative stimuli (p 's $> .05$).

The appetitive stimuli (*DSchoc*, *DScrisp*, Pavlovian *CS+*) were collapsed to investigate whether rewarding stimuli, regardless of type of conditioning type, produced different activation in comparison with neutral and non-rewarding stimuli (*DS-*, Pavlovian *CS-*), again irrespective of conditioning type. T-tests (Rewarding stimuli > Non-rewarding stimuli) suggested a trend that the rewarding stimuli activated areas previously associated with reward, such as the striatum in the left hemisphere ($p = .080$) and the insula bilaterally ($p = .075$). T-tests (Non-rewarding stimuli > rewarding stimuli) showed that the non-rewarding stimuli did not produce greater activation in any regions compared to the rewarding stimuli (p 's $> .05$).

BOLD responses to the *DSchoc* and *DScrisp* stimuli were separately contrasted with the Pavlovian *CS+* to view the different effect rewarding stimuli have depending on conditioning method. T-tests (*DSchoc* > Pavlovian *CS+*) revealed that *DSchoc* produced greater bilateral activation in the striatum ($p = .001$), and left hippocampus ($p = .006$) compared to the Pavlovian *CS+*. T-tests (Pavlovian *CS+* > *DSchoc*) also showed that the Pavlovian *CS+* did not produce greater activation or deactivation in comparison (p 's $> .05$). T-tests (*DScrisp* > Pavlovian *CS+*) comparisons showed that *DScrisp*, however, produced greater bilateral activation in the striatum and putamen (p 's $< .001$) compared to the Pavlovian *CS+*. T-tests (Pavlovian *CS+* > *DScrisp*) showed that the Pavlovian *CS+* did not produce greater activation than the *DScrisp* stimulus.

Similarly, BOLD responses to the *DS-* were compared to the Pavlovian *CS-* to view the effect non-rewarding stimuli have depending on conditioning type. T-test (*DS->*Pavlovian *CS-*) showed that the *DS-* produced greater bilateral activation in the insula ($p < .001$), amygdala ($p < .001$), OFC ($p < .001$), and striatum ($p < .001$). It also produced unilateral activation in the right putamen ($p = .005$), and left hippocampus ($p = .016$) compared to the Pavlovian *CS-*. T-tests (*CS->**DS-*) also revealed that the *CS-* did not produce unique activation or deactivation in any region compared to the *DS-* (p 's $> .05$).

4.3.1.5. Contingency Awareness.

To test whether awareness of the conditioned stimulus and food association affected BOLD responses to the conditioned stimuli a between-measures (Contingency Awareness: Aware, Unaware) t-test was performed on BOLD responses to the stimuli in the activated regions shown by the main analysis. There was no difference in activation in any of the regions of interest between aware and unaware participants in response to the Pavlovian *CS+* (aware $n = 7$, unaware = 9), Pavlovian *CS-* (aware = 3, unaware = 13), *DSchoc* (aware $n = 9$, unaware = 7), *DSchoc* (aware $n = 9$, unaware = 7), and *DS-* (aware $n = 12$, unaware = 4) (p 's $> .05$).

4.3.1.6. Gender Differences.

To test whether males and females differed in their BOLD responses to the conditioned stimuli a between-measures (Gender: males, females) ANOVA was performed on BOLD responses to all of the individual stimuli. There were no significant voxels that were activated differentially between males and females to the conditioned stimuli (p 's $> .05$).

4.3.1.7. Neural activity and behavioural correlates.

To test whether performance in the discriminative stimuli task affected neural responses correlations were performed between regions shown to be significant in response to each stimuli and behavioural results. Winning more chocolate rewards in response to the *DSchoc* produced less bilateral activation in the hippocampus ($r(13) = -.51$, $p = .054$). More key presses performed in response to the *DS-* produced less bilateral activation in the hippocampus in response to the *DSchoc* ($r(13) = -.55$, $p = .035$).

In regards to Pavlovian stimuli, there were no significant correlations their pleasantness rating of the 10% sucrose and any activation or deactivation in the regions of interest (p 's $> .05$).

4.3.1.8. Neural activity and participant characteristics.

To test whether individual differences, such as restraint and disinhibition scores, were related to neural activity, correlations were performed. Restraint scores were significantly positively correlated with activity in the hippocampus in response to the *DSchoc* ($r(13) = .57, p = .028$), and in the striatum in response to the *DScrisp* ($r(13) = .73, p = .002$); higher restraint individuals had greater bilateral activation in both these areas. Activity in the hippocampus in response to the *DSchoc* was also correlated with participants' subjective appetite rating prior to conditioning ($r(13) = -.62, p = .014$) so that higher appetitive rating individuals had less bilateral activation. There were no significant correlations between BMI and disinhibition scores and any activation or deactivation in any of the regions of interest ($p's > .05$).

Disinhibition and BMI were highly and positively correlated ($r(13) = .48, p = .058$). Pleasantness ratings of the 10% sucrose solution was, also, positively correlated with BMI ($r(13) = .78, p < .001$).

4.3.2. Behavioural Analysis.

Subjective appetite rating prior to conditioning was unrelated to total presses participants performed on the discriminative stimuli task ($r(13) = .56, p = .290$). BMI was, also, unrelated to total presses performed during this task ($r(13) = .18, p = .523$). See Table 4.7.

There was no significant difference between the total number of presses to the two different *DS*- stimuli ($t(14) = -.73, p = .480$). Thus the two *DS*- stimuli were collapsed. Participants pressed the keys significantly more to the *DS*- than to the *DSchoc* ($t(14) = -4.44, p = .001$). They also pressed significantly more to the *DS*- than the *DScrisp* ($t(14) = -4.92, p < .001$). There was no difference in total presses between the *DSchoc* and *DScrisp* ($t(14) = -.85, p = .412$).

To test whether awareness of the stimulus-reward association affected behaviour in the discriminative stimuli task, t-tests were performed to see whether contingency awareness (aware, unaware) affected key presses during the task. The results showed that awareness of the contingency between the stimulus and its associated reward in discriminative stimuli task differentially affected the operant behaviour. Aware participants (282 ± 44) pressed the key significantly more when the *DSchoc* was on screen compared to unaware participants (123 ± 39) ($t(13) = 2.76, p = .016$). There was no difference between awareness conditions for the other stimuli ($p's > .05$).

Table 4.7. Participant characteristics.

Characteristic	Mean (SEM)
BMI	23.39 (.82)
Restraint	3.00 (.47)
Disinhibition	5.80 (1.01)
Hunger rating prior to conditioning	40 (8)

4.4. Discussion

The aim of the current experiment was to investigate the effects of Pavlovian and discriminative stimuli associated with food on BOLD responses upon stimulus presentation in an MRI scanner.

One key finding was that a cue paired with a sweet taste (Pavlovian CS+) produced deactivation in areas previously associated with reward, such as the striatum, putamen and insula. Although previous research has implicated similar regions in response to food (Small et al., 2003; Babbs et al., 2013) and food cues (O'Doherty et al., 2004), these studies have shown activation rather than deactivation. In contrast a cue paired with a neutral taste (Pavlovian CS-) produced deactivation in the amygdala. There was no significant difference between the two stimuli in regards to regions activated suggesting that the individual effects they produced were relatively small effects.

The observed deactivation in reward areas may be due to the methodology used. In this study taste-associated Pavlovian stimuli were presented in the MRI scanner however the expected taste was not received. Previous research (Morris et al., 2012) has shown deactivation in the ventral striatum in response to unexpected reward omission. The observed deactivation in the present study could be due to not receiving the expected associated taste in the scanner. However, deactivation is a complicated finding to interpret in regards to fMRI (Frankenstein et al., 2003). It remains unknown whether the deactivation observed is due to neural activation downstream of a particular region or active inhibition. Previous research (Ghatan, Hsieh, Petersson, Stone-Elander, & Ingvar, 1998) has shown region specific deactivation in areas deemed functionally irrelevant or detracting. However, in regards to the present study, it remains unknown why only Pavlovian stimuli caused specific deactivation, especially in regions previous research has shown to be activated. It should be noted though that research has shown that BOLD signal deactivation can simultaneously have neuronal spiking (Harel, Lee, Negaoka, Kim, & Kim, 2002).

Another key finding was that a cue associated with a chocolate reward if the correct response was produced (*DSchoc*) activated the hippocampus. A cue associated with a crisp reward dependant on response produced (*DScrisp*) activated the striatum and thalamus. A discriminative cue not associated with obtaining a reward (*DS-*) did not activate any of the regions of interest. When collapsed the rewarding appetitive stimuli activated the striatum (an area associated with reward) whereas the non-rewarding discriminative stimuli activated the *DS-*.

The difference between discriminative and Pavlovian stimuli was also an interesting comparison. The discriminative stimuli, regardless of associated reward, activated areas associated with reward, such as the striatum, putamen, and insula, as well as the amygdala and hippocampus to a greater extent than the Pavlovian stimuli. However, the Pavlovian stimuli didn't activate or deactivate any regions more than the discriminative stimuli. This would seem to suggest that the discriminative stimuli is an addition of information onto Pavlovian stimuli.

Explicit knowledge of the stimulus-outcome association did not affect neural activation for any of the stimuli. Whilst this contradicts previous research (Birch et al., 1989), it again supports behavioural findings from our laboratory highlighting the possible lack of necessity for awareness in specialist systems, such as the gustatory system (Brunstrom, 2004).

However, caution must be taken with the interpretations of the comparisons between the rewarding discriminative and Pavlovian stimuli. The appetitive stimuli for the Pavlovian and discriminative stimuli signal qualitatively different rewards. The discriminative associated rewards are an image of chocolate and crisps, whereas the Pavlovian *CS+* is associated with a sweet taste. This means they may have differed on a number of properties such as whether the reward was an oral experience or a hypothetical reward, caloric value of the reward, or hedonic value (whilst pleasantness ratings of the 10% sucrose solution was obtained, ratings were not obtained for chocolate and crisps). Future studies could replace the image of chocolate/crisps with a taste of a sweet taste if the correct response is produced upon presentation of a discriminative stimulus. This would specifically address the difference, neurologically, between discriminative and Pavlovian stimuli. Similarly, caution must be taken with the non-rewarding stimuli (*DS-*, *CS-*) due to one being non-rewarding and one being associated with a neutral taste.

In conclusion, although minor limitations may limit the conclusions drawn when directly comparing the Pavlovian and discriminative stimuli, this methodology provides a novel

and useful way of investigating the neural regions activated and deactivated by conditioned stimuli.

4.5. Tables.

Table 4.1. Effects of discriminative stimulus presentation.

Stimuli	X	Y	X	Z value	k	(De)Activation
<i>DSchoc</i>						
Hippocampus	-18	-34	-2	3.50	4	↑
<i>DScrisp</i>						
Striatum	20	-22	20	4.42	9	↑
Thalamus	22	-14	12	4.42	8	↑
<i>DS-</i>						
None						
Pavlovian CS+						
Striatum	-4	8	-6	3.78	21	↓
	14	20	-2	3.34	2	↓
Putamen	-24	0	12	3.71	6	↓
Insula	-32	12	-16	4.01	15	↓
Pavlovian CS-						
Amygdala	-26	-2	-22	3.51	3	↓
Thalamus	-4	-14	2	3.69	9	↓

Table 4.2. Comparison of stimuli (within conditioning types).

Stimuli	X	Y	X	Z value	k	(De)activation
Pavlovian CS+ v Pavlovian CS-						
None						
DSchoc v DScrisp						
None						
DScrisp > DS-						
Striatum	-10	-2	18	3.63	16	↑
DS- > DSchoc						
Middle OFC	20	60	-12	4.05	14	↑
	-28	28	-18	3.99	8	↑
Thalamus	-20	-20	10	3.53	4	↑
DScrisp > DS-						
Hippocampus	-24	-26	-10	3.69	9	↑
DS- > DScrisp						
OFC	26	36	-20	3.88	47	↑
	-6	66	-8	3.70	9	↑

Table 4.3. Comparison between general discriminative and Pavlovian stimuli.

Comparison	X	Y	Z	Z value	k	(De)activation
Discriminative > Pavlovian						
Amygdala	-26	-6	-14	4.23	25	↑
	26	0	-12	3.49	17	↑
Striatum	28	2	8	4.22	84	↑
	-18	0	24	4.22	289	↑
Putamen	28	2	8	4.41	84	↑
	-16	10	-2	3.79	57	↑
Hippocampus	-26	-8	-16	3.80	14	↑
	32	-16	-12	3.53	9	↑
Insula	-28	20	-20	3.77	9	↑
	42	6	2	3.41	8	↑
Pavlovian > discriminative						
None						
General CS+ > General CS-						
Insula	-40	-18	-4	3.33	3	↑
	38	-2	4	3.27	3	↑
Striatum	-30	-8	2	3.76	12	↑
General CS- > General CS+						
None						

Table 4.4. Comparison between discriminative stimuli.

Comparison	X	Y	Z	Z score	k	(De)activation
DS+ > DS-						
Striatum	-10	-2	18	3.84	20	↑
DS- > DS+						
OFC	26	36	-20	3.99	64	↑
	-6	68	-6	3.69	5	↑

Table 4.6. Comparison between non-rewarding discriminative stimulus and Pavlovian CS-

Comparison	X	Y	Z	Z value	k	(De)activation
DS- > Pavlovian CS-						
Putamen	28	6	10	4.69	103	↑
	-16	10	2	3.11	1	↑
Insula	-28	20	-20	4.50	22	↑
	42	22	-8	3.82	18	↑
Amygdala	-24	-6	-16	4.34	28	↑
	26	4	-20	3.51	15	↑
OFC	-28	24	-18	4.32	22	↑
	42	44	22	4.18	16	↑
Striatum	28	6	10	4.69	103	↑
	-10	18	12	3.58	7	↑
Hippocampus	-26	-8	-16	3.97	15	↑
Pavlovian CS- > DS-						
None						

Chapter 5: General Discussion

5.1. Introduction.

Exogenous food-associated stimuli can potentiate consumption of palatable foods in the absence of immediate nutrient needs, potentially promoting overconsumption and contributing to the global obesity epidemic. However, the majority of research investigating Pavlovian stimulus-reward associations in humans has failed to control the stimulus-reward associations by investigating existing stimulus-reward pairings. Different amounts of stimulus-reward pairings have been shown to affect ingestive behaviour (Valle, 1968) and neurological structure (Maren, 1998; 1999) and thus initially neutral stimuli must be used in order to control contingencies. Additionally, past research has failed to account for individual differences, such as restraint (Brunstrom et al., 2004), which can influence learning (Fedoroff et al., 1997). The majority of previous research has also failed to distinguish between Pavlovian stimulus-reward associations and (discriminative) stimuli, which signal the opportunity to obtain a reward if a certain behaviour is produced. These two types of stimuli are behaviourally (Di Canio & Everitt, 2003) and neurologically (MacDannald et al., 2005) distinct. To our knowledge there has been no investigation into the effects of discriminative stimuli on food intake as the previous research outlined above (Chapter One) tended to focus on the role of Pavlovian stimuli in eating behaviour. Both types of food-associated stimuli may be contributing to overconsumption, but how remains unknown. This thesis set out to explore the effects of Pavlovian and discriminative stimuli on food intake behaviour in a controlled and rigorous manner by using novel and initially arbitrary stimuli and conditioning certain rewards to the stimuli.

The following discussion will initially provide an overview of the main behavioural and neural findings, discussed in Chapters 2, 3, and 4, and address some of the limitations, theoretical implications and suggestions for future research.

5.2. Main findings: a summary

5.2.1. Study One: Cue-potentiated feeding by sweet-associated Pavlovian stimuli

Chapter One investigated the effects of sweet-associated Pavlovian stimuli on subsequent *ad libitum* intake. The key, and hypothesised, finding from Study One of this chapter was that a stimulus previously associated with a sweet taste increased consumption, specifically of sweet

food items, compared to a cue paired with a neutral taste when presented in a snack test. However, this evidence was limited by the lack of an appropriate 'no cue' control. Without this control it is unclear whether the observed cue-potentiated intake was due to a suppression of consumption in the presence of the neutral-paired stimulus. Contrary to our hypothesis, there was no evidence that cue-potentiated feeding was affected by the participants' explicit level of awareness of the stimulus-taste association. Additionally, and contrary to past research, the sweet-paired stimulus did not significantly influence the latency to the first food selected, or which food item was selected first. The Pavlovian stimuli did not influence hedonic or sensory evaluations of the snack foods.

5.2.2. Study Two: Cue-potentiated feeding by sweet-associated Pavlovian stimuli in sweet-liking and disliking individuals

Study Two investigated whether it was the hedonic component of the sweet taste that drove the cue-potentiated feeding seen in Study One by recruiting sweet-likers and sweet-dislikers. The results produced contrasting findings from Study One; specifically, there was no evidence of cue-potentiated feeding in general or sweet-specific intake. The participants in the *CS+*, *CS-* and *No Cue* conditions consumed the same amounts of the snack foods provided. Importantly, the sweet-likers (who fit the same specifications as those in Study One) did not show cue-potentiated feeding. However, It may be that Study Two was simply underpowered to find an effect; only ten sweet-likers were assessed in the *CS+* condition in Study Two as compared to 22 in Study One. Alternatively, the lack of replication may be due to the generally low intake across participants. When those participants who ate less than 283 calories of snacks were removed from the analysis a significant cue-potentiated feeding effect was observed in the *CS+* condition. Study Two did replicate the finding that contingency awareness did not affect consumption, providing more evidence for the lack of necessity for explicit contingency awareness to affect behaviour. The sweet-associated stimuli also did not affect the hedonic or sensory evaluations of the test snack foods. Again there was no significant difference between conditions in latency to first food selected.

5.2.3. Study Three: The effect of discriminative stimuli on intake

Whilst the previous two studies investigated the effects of Pavlovian stimuli on intake, Study Three looked to investigate the effect of discriminative stimuli on intake. Contrary to hypothesis, the study provided strong evidence that stimuli associated with earning no rewards decreased intake compared to stimuli associated with the opportunity to win chocolate rewards if participants produced the correct response. However, unlike the findings

from Study One and past research, this decrease was not food-specific. Similar to the Pavlovian stimuli, participants' explicit knowledge of the association between the discriminative stimuli and reward did not affect consumption. Discriminative stimuli had no effect on hedonic or sensory evaluations of the snack foods. There was no evidence that discriminative stimuli affected the latency to first food selected or influence which food was first selected.

5.2.4. Study Four: The effect of discriminative stimuli on intake whilst sated

Study Four wanted to investigate whether the key findings from Study Three persisted whilst participants were sated. Whilst the exact findings (cue-suppression whilst non-sated) from Study Three were not replicated, there was evidence of a gender-specific cue effect. Regardless of hunger state, males did not differ in snack consumption between conditions; however females in the *DS+* condition (a stimulus associated with obtaining a chocolate reward) ate significantly more than females in the *DS-* condition. This supports the findings from Study Three, although Study Three did not show a gender-specific effect. This cue-potentiated by only females was, also, cue-specific; the *DS+* condition eating more chocolate than the other conditions (groups did not differ on consumption of the other snack food items). The lack of necessity of contingency awareness to affect intake was supported again. Discriminative stimuli had no effect on hedonic or sensory evaluations. The stimuli had no effect on which food was selected first or latency to that first selection.

Table 5.1. Summary of key findings from behavioural Studies One-Four within the thesis.

Study	Cued effect on intake?
Study One	Yes – increased intake by cue associated with a sweet-taste
Study Two	No – findings in predicted direction when low-intake participants excluded
Study Three	Yes – decreased intake by cue associated with not obtaining a reward
Study Four	No – although significant effect for females

5.2.5. Study Five: brain regions underlying neural responses to Pavlovian and discriminative stimuli

Study Five looked to expand upon the findings from Study One and Three, by investigating the brain regions underlying neural responses to a Pavlovian and discriminative

stimuli conditioned in the same manner as in Study One and Three respectively. Study Five provided strong evidence for the *CS+* (a Pavlovian stimulus associated with a sweet taste) to produce bilateral deactivation in reward-associated areas, such as the striatum, putamen, and the left insula. The *CS-* (a Pavlovian stimulus associated with a neutral taste) produced deactivation in the left amygdala and left thalamus. The *DSchoc* (a discriminative stimulus associated with chocolate if the correct behaviour was produced) produced bilateral activation in the hippocampus, and the *DScrisp* (a discriminative stimulus associated with crisps if the correct behaviour was produced) activated the right striatum and right thalamus. The *DS-* (a discriminative stimulus associated with no rewards) did not activate any regions of interest. There was no evidence for contingency awareness to produce differential activation. Contrary to Study Four there was no evidence of a gender difference.

5.3.1. Theoretical implications.

The discussions in chapters 2-4 have partly explored the theoretical implications for the studies individually. This section will consider the implications from the thesis as a whole; specifically, the consistency of the findings across studies, the necessity of contingency awareness to affect behaviour and how the food-associated stimuli influence food intake.

5.3.2. The lack of consistency of cue-potential feeding

A key issue that has been emphasised in this thesis is the lack of consistency of the cue-potential findings reported in Chapter Two and previous research discussed in Chapter One. The lack of consistency is not just between the replication of the Pavlovian studies (Study One and Two) but also in the cue-suppression finding in Study Three, which goes against the hypotheses and the cue-potential findings in Chapter Two. Whilst the reliability of cue-potential feeding effects from past research could be questioned, the lack of consistent evidence from the present thesis and previous research may be due to differences in methodology of the intake test used. The majority of past research has used extended intake sessions ranging from ten minutes (Fedoroff et al., 1997), 15 minutes (Birch et al., 1989) to no limit (Cornell et al., 1989). In comparison, the present thesis used a five minute eating period. If, as our data seems to show, the conditioned stimuli used in this thesis does not have a significant effect initially (shown by the lack of evidence for conditioned stimuli to influence latency or first food selected) but a prolonged effect, this suggests that the longer the eating period the greater the effect shown. Studies using a longer intake period (such as Fedoroff et al., 1997) have found consistent findings, so it may be possible that the shorter intake period

used in this thesis limited the cues subtle effects. This might have been particularly true of Study Two where inadequate snack consumption appeared to mask the different effects of the cues on intake. Thus, future research should investigate this by using the current methodology but varying the length of the test intake periods to determine whether this impacts amount of snacks consumed in response to a cue.

Despite the contrasting findings, there are a number of conclusions we can draw from this thesis. Firstly, although a strong effect when observed, conditioned consumption seems to be affected by a large number of variables, such as gender and low overall intake. Additionally, conditioned stimuli, regardless of form of conditioning, seem not to affect hedonic or sensory evaluations of the foods presented in the intake test. According to the incentive salience theory (Robinson & Berridge, 1993), food-associated stimuli would not be predicted to affect liking ratings; specifically, the stimuli would affect wanting. Whilst throughout the thesis we found no evidence for the stimuli to affect participants' explicit wanting it may be that such an explicit approach to measuring wanting may provide an accurate measure. This is somewhat unsurprising as 'wanting', as defined by Robinson and Berridge (1993), is the unconscious process that underlies conscious wanting. Explicit measures, such as the VAS ratings used in the present thesis, may not measure this unconscious process (Finlayson et al., 2007b). Berridge and Robinson (1998) suggested that implicit 'wanting' is best measured by voluntary intake or preference tests. Whilst this thesis provided some evidence for conditioned stimuli to influence intake, the stimuli did not influence which food was selected, the latency to the food selected, or preference for the test snack foods. The lack of evidence for these phenomena, and the inconsistency of cue-potentiated feeding findings, may be explained by incentive sensitization theory (Robinson & Berridge, 1993). Incentive salience theory suggests that in drug addicts the hijacking of the dopamine reward system by drugs of abuse leads to a sensitization of this system which causes drug-associated stimuli to become salient and wanted (Berridge & Robinson, 2003). This dopamine reward system is the same system responsible for motivation to natural rewards, such as food or sex. However, whether food causes these neural changes is debateable (Volkow, Wang, Tomasi, & Baler, 2013; Pelchat, 2009; See Rogers & Smit, 2000). It is possible that in regards to eating behaviour, and without these neural adaptations, other factors play a more prominent role. For example, compared to the subtle conditioned stimuli used throughout the present thesis, it is possible that other variables had a greater influence on behaviour such as first food selected; for example social norms (e.g. selecting savoury foods first; Chapter Three).

Despite this, the elusive nature of stimulus-potentiated feeding may not be too surprising considering the inconsistent findings from research investigating evaluative

conditioning using visual stimuli. The most consistent evaluative conditioning findings have been found by the Leuven group (who defined the classic evaluative conditioning face-pairing paradigm) (Stuart, Shimp, & Engle, 1987), however, Rozin, Wrzesniewski and Byrnes (1998) highlight that their effect size has substantially reduced across studies. Similarly, using flavour-flavour conditioning, Rozin et al. (1998) found that the statistical significance of their findings was not replicated consistently. They summarised that the boundary conditions of evaluative conditioning, and thus flavour-flavour and flavour-nutrient learning, are yet to be fully understood. Similarly, whilst some studies have shown consistent flavour-nutrient learning, others have failed to find such evidence (see Yeomans, 2012). Conducting laboratory experiments, such as those conducted throughout the present thesis, may reduce the effect of evaluative conditioning or make it more sensitive to parameters that can vary within and between experiments (Rozin et al., 1998).

The studies in the present thesis may have suffered from similar issues to the aforementioned evaluative conditioning research. For example, if evaluative conditioning is driving the stimulus-potentiated feeding in experiment 1 then the lack of replication (or possible small effect size) may replicate other research in the field. However, this theory is speculative as subjective liking ratings of the stimuli (and thus any change in liking observed) were not measured.

5.3.3. Contingency awareness

Contingency awareness is the knowledge that a specific stimulus predicts a specific outcome (Lovibond & Shanks, 2002). Birch et al. (1989) indicated that explicit awareness was critical for cue potentiation of eating. However, the certainty of this idea is far from assured. Throughout this thesis we found no evidence that awareness of the stimulus-outcome relationship affects behaviour (i.e. total consumption, or hedonic and sensory evaluations of the test foods). Birch et al.'s (1989) finding is also contradicted in the gustatory literature (Baeyens, Eelen, Van den Bergh, & Crombez, 1990) and other fields (Ruys & Stapel, 2009).

The contradictory nature of research into contingency awareness may depend on the methodology of the studies investigating it. Baeyens et al. (1990) paired flavours or colours with sugar or tween and measured contingency awareness by presenting the flavour or colour and asking which taste (sugar or tween) was presented with it most of the time. They found that contingency awareness was needed for colour-flavour conditioning but not for flavour-flavour conditioning. However, participants were explicitly told some of the drinks contained tween, sugar and some merely fruit flavours. This prompting of predictive significance

considerably alters the number of contingency aware participants (Hogarth, Dickinson & Duka, 2005). Dickinson & Brown (2007) addressed this problem in their colour-flavour paradigm utilising blocking (when a previous learned stimulus-outcome blocks the learning of a new stimulus when in combination with the previous stimulus). For each taste participants rated their level of certainty that the colour had been presented with each flavour. This overcame the problem produced by measuring taste expectancy at the moment the stimulus was presented, which can affect the stimulus-taste relationship as it draws specific attention to this relationship. They found that flavour-evaluative conditioning occurred independently of contingency awareness. This finding is supported by Lovibond and Shanks (2002) who suggested that awareness might not necessarily be required for conditioned performance. In their review they conclude that behaviour without awareness is extraordinary and most examples of classical conditioning require awareness. However, conditioning without awareness may occur within specialist systems, such as the gustatory system, with the conditioning of taste properties one example (Stevenson et al., 1995; Stevenson, Boakes, & Prescott, 1998). Why certain specialist systems may not require awareness remains unknown, however, the learning of the consequences of tastes is crucial for organisms. Kalat and Rozin (1973) showed that even when long delays between taste and post-ingestive consequence occur, the relationships are still learned. The consequences (for example, illness or death) of certain tastes are too important to require explicit awareness; it is more important to learn unconsciously than not at all. Although this may be the reason the gustatory system does not require awareness during conditioning, it remains as speculation.

5.4. Future experiments

One of the major limitations, as discussed in Chapter Four, when comparing the Pavlovian and discriminative stimuli is that the stimuli signalled very different rewards; the Pavlovian stimuli signalled an experience of tastes, whereas the discriminative stimuli signalled the receipt of visual rewards. To truly investigate the differences between the two types of learning a yoking procedure could be used. A yoked control design involves pairing two animals so that the behaviour of one determines the reward receipt of the other (Church, 1964). Specifically, one of the pair serves as the experimental subject and the other as the control subject. The experimental subject is placed in an instrumental learning situation; whereas the control subject is placed in a similar situation though reward is determined by the experimental subject's response. Using the methodology from Chapter Three, one participant could perform the usual instrumental conditioning procedure whilst another merely watches

their screen (Pavlovian learning occurring whilst watching the stimuli being associated with different rewards). After these two types of conditioning participants could then be given a snack intake test or placed in an MRI scanner to compare the two types of learning in a rigorous and controlled manner. Whilst this has been investigated to an extent in an fMRI setting (O'Doherty et al., 2004), this has yet to be investigated in ingestive behaviour studies.

The ability of a discriminative stimulus associated with earning no rewards (DS-) to reduce food intake, shown in Study Three, could be exploited as a means of refusing intake in people who desire to lose weight. However, future research should consider and address a number of limitations in the present study before applying the current findings to restricting one's calories. Firstly, the current study only used low restraint individuals who by definition have less intention to diet, achieve, or maintain a desired body weight (Stunkard & Messick, 1985; Laessle, Tuschl, Kotthaus, & Prike, 1989). To determine whether cues associate with no-rewards can be used to reduce intake, this study should be replicated using individuals with a higher level of dietary restraint and/or who are currently dieting to lose weight. Similarly, we would need to determine whether a similar account of cue-potentiated feeding effects are seen in the overweight or obese, as the present study only used normal-weight individuals. However, a benefit of the methodology applied was the use of high-calorie, high fat snack foods; energy-dense foods that may contribute to obesity. The cue-attenuation finding in Chapter Three then has great applicability in potentially aiding reduction of the foods most likely to be contributing to obesity.

Findings from Chapter Two showed cue-potentiation of snack foods by Pavlovian stimuli associated with a non-ingested hedonic taste. This suggests that, in this instance, all that is needed for conditioning to occur is a hedonic experience. In an obesogenic environment the potential for conditioning is substantial. Future work should investigate whether cue-potentiated feeding can occur for low energy dense foods. If stimuli associated with a hedonic experience can increase fruit or vegetable consumption then the implications are considerable. For example, findings from Study One suggested that cues associated with a sweet taste increased consumption of sweet snacks foods. It would be interesting to investigate whether the sweet taste elicits potentiated consumption solely of snacks foods or could applied to sweet foods generally, such as sweet low energy dense foods (i.e. most fruits).

A key finding from Chapter Four was the deactivation of brain reward regions in response to Pavlovian stimuli associated with a sweet taste. As discussed in Chapter Four one

possible reason for this deactivation (as opposed to the hypothesised activation in the same areas) could be the absence of a sweet taste whilst the stimulus was presented in the fMRI scanner. Future work should address this by delivering tastes to participants during stimulus presentation. The reason for this is critical; if people experience a deactivation in brain reward areas upon presentation of food-associated stimuli when no reward is received then people may try to seek reinforcers in order to activate these reward circuits (Wang, Volkow, Thanos, & Fowler, 2004). The same would apply if Pavlovian stimuli simply cause deactivation in brain reward areas regardless of reward receipt. However, more research is needed to confirm these possibilities. Additionally, as some research has suggested that reductions in dopamine receptor availability are inversely correlated with body weight (Wang, Volkow, & Fowler, 2002; Volkow et al., 2008), the interaction between obesity and neural responses to food-associated stimuli warrants further investigation.

Similarly, Chapter Four merely investigated the effects of these stimuli on neural responses without investigating whether these responses, such as the deactivation in the striatum, translated to consumption changes in a snack intake test. Whilst this is a limitation of the study, methodologically investigating this would be difficult. This is because presenting the stimuli in the fMRI scanner may serve as an extinction session so conducting a snack test post-scan would not be indicative of initial conditioning prior to extinction. Additionally, conducting the snack test prior to the scan would be problematic as the intake test may serve as a new conditioning session in which the stimuli are associated to all the foods in the test. One possible solution to this methodological issue may be to conduct the conditioning whilst in the scanner and the snack test post-scan.

The current thesis also included only one five-minute eating session; it is not known whether these cues would continue to have an effect over multiple sessions (though as each intake test would theoretically serve as an extinction session it would be highly doubtful that the effect would be observed continually). Weingarten (1983) showed cue-potentiated feeding within the test but showed animals did compensate for this increased intake over 24 hours by later reducing their intake. It remains unknown whether any of the effects observed in this thesis last beyond one eating period.

Whilst this thesis provides some evidence for conditioned stimuli to affect consumption, the specific reason underlying the phenomena remains unknown. According to Robinson and Berridge's (1993) incentive salience theory after pairing with a hedonic reward the stimuli becomes a motivational magnet, increasing wanting for the stimulus and inducing

behaviour in the stimulus' presence. However, this remains theoretical. As discussed in Chapter One, there have been investigations into the underlying mechanisms of ingestive behaviour, specifically wanting and liking. For example, the Leeds Food Preference Questionnaire (LFPQ) has been shown to be sensitive to individual eating traits, such as disinhibition (Finlayson, Bordes, Griffioen-Roose, de Graaf, & Blundell, 2012). However, the methodology has not been used to investigate the effect of conditioned stimuli on wanting and liking. Pavlovian stimuli associated with a sweet and a neutral taste could be presented to see if the conditioned stimuli increase implicit wanting as predicted by the incentive salience theory. Whilst the current thesis investigated implicit wanting by measuring voluntary intake and preference (Berridge & Robinson, 1998), perhaps a measure less influenced by social norms, amongst other factors, may reveal the psychological mechanisms underlying cue-potentiated feeding in humans.

5.5. Conclusions

The present thesis aimed to investigate the effects of Pavlovian and discriminative stimuli on intake in a free-feeding situation, and the brain regions underlying these behaviours. There was evidence of Pavlovian stimuli associated with a sweet taste potentiating feeding, specifically of sweet foods, compared to stimuli associated with a neutral taste. Additionally, discriminative stimuli associated with winning no rewards suppressed overall intake compared to the stimuli associated with winning chocolate. However, replication of both of these experiments did not produce the same findings, although a novel gender-cue interaction and low consumption generally may have complicated the results. The studies conducted also provided evidence for Pavlovian stimuli to deactivate brain reward regions, such as the striatum, whilst discriminative stimuli activated regions such as the hippocampus, striatum, and thalamus. The longevity of the behavioural and neural effects found in the experimental work of this thesis warrant further investigation. In particular it would be important to see whether the effects of these conditioned stimuli are compensated for during the following 24 hours post-learning. Despite limitations, the present thesis provides novel and interesting methodology to investigate the effects of conditioned stimuli in human appetitive research.

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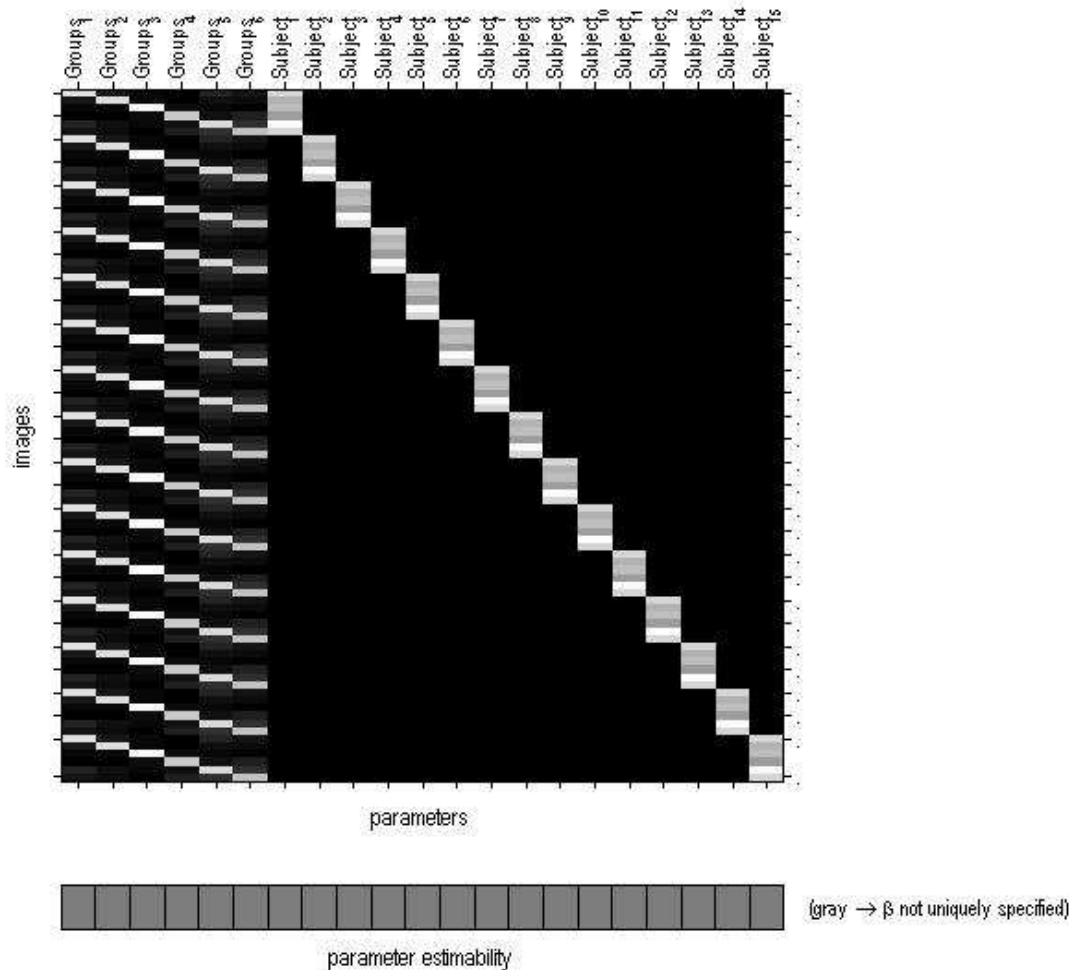
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Appendix 4.1. fMRI matrix design.

Statistical analysis: Design



Design description...

Design : ANOVA - within subject
Global calculation : omit
Grand mean scaling : <no grand Mean scaling>
Global normalisation : <no global normalisation>
Parameters : 6 condition, +0 covariate, +15 block, +0 nuisance
 21 total, having 20 degrees of freedom
 leaving 70 degrees of freedom from 90 images